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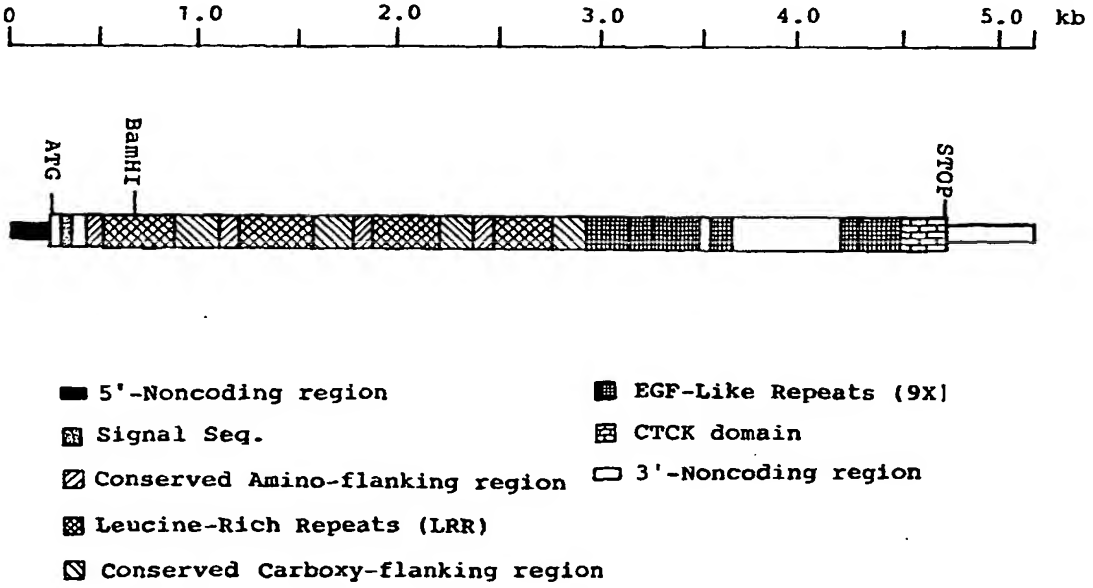

(57) Abstract

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptides of the present invention are human *slit* polypeptides. The invention also relates to identifying mesenchymal stem cells (MSCs) or other cells comprising such polypeptides or polynucleotides that encode the polypeptides.

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Human Slit Polypeptide and Polynucleotides Encoding Same

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptides of the present invention are human Slit polypeptides. The invention also relates to identifying mesenchymal stem cells (MSCs) or other cells comprising such polypeptides or polynucleotides that encode the polypeptides.

Proteins containing epidermal growth factor (EGF)-like sequences have been shown to play an important role in many aspects of eukaryotic cell control, acting as signals for proliferation, growth inhibition, and differentiation. A common feature of these proteins is their involvement in extracellular events and ligand-receptor interactions. In characterizing genomic DNA identified by cross-hybridization to the sequence coding for the tandem EGF repeats of Notch in Drosophila, a related gene sequence from an unlinked locus that also has EGF repeats was discovered. Isolation and characterization of it, showed a corresponce to the slit

locus. Further characterization of the related gene sequence established that null mutations to it would result in disruptions of the embryonic central nervous system (CNS) (Rothberg et al. 1988). Thus, it was shown to be involved in neurogenesis.

The Drosophila slit protein contains two types of repeated amino acid sequences: leucine rich repeats ("LLR") and epidermal growth factor-like repeats ("EGF"). Its LRRs are arranged in four groups, each composed of four or five LRRs surrounded by conserved amino- and carboxy-flanking regions. The presence of both the LRRs and EGF-like repeats within a single protein make slit unusual in that such combination is not found in any other type of known protein. The absence of any potential transmembrane domains in a sequence having a typical signal sequence and two known extracellular-associated motifs suggests that the slit locus encodes a secreted extracellular protein. The LRR regions of the slit protein and such regions of related proteins participate in extracellular protein-protein interactions. Further, the EGF areas of the slit protein and such regions of related proteins participate in extracellular protein-protein reactions. Moreover, the slit protein is synthesized and secreted by midline glial cells can be come associated with axons. Among other functions, it influences the differentiation of midline cells from the neuroepithelium.

In accordance with one aspect of the present invention, there are provided novel polypeptides and polynucleotides, more particularly, the polypeptides of the present invention are of human origin and are found in human mesenchymal stem cells. MSCs are the formative

pluripotential blast cells found inter alia in bone marrow, blood, dermis and periosteum that are capable of differentiating into any of the specific types of mesenchymal or connective tissues (i.e. the tissues of the body that support the specialized elements; particularly adipose, osseous, cartilaginous, elastic, and fibrous connective tissues) depending upon various influences from bioactive factors, such as cytokines. The polypeptide is designated as human Slit. The human Slit polypeptide according to the present invention, as well as biologically active and diagnostically or therapeutically useful fragments, analogs and derivatives thereof, is of use in studing the culturing of MSCs and detection of their differentiation and development into multipotent cells.

In accordance with another aspect of the present invention, there are provided isolated nucleic acid molecules encoding such polypeptides, including mRNAs, cDNAs, genomic DNA as well as biologically active and diagnostically or therapeutically useful fragments, analogs and derivatives thereof.

In accordance with another aspect of the present invention there are provided nucleic acid probes comprising nucleic acid molecules of sufficient length to specifically hybridize to sequences of the present invention.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptides by recombinant techniques which comprises culturing recombinant prokaryotic and/or eukaryotic host cells, containing a nucleic acid sequence

of the present invention, under conditions promoting expression of said protein and subsequent recovery of said protein.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptides, or polynucleotides encoding such polypeptides for identifying human MSCs by utilizing the polynucleotides as probes or by expressing the polypeptides encoded thereby, using such polypeptides to produce an antibody specific for one of the polypeptides and then utilizing the antibody to identify the MSCs. Further such polynucleotides, polypeptides and antibodies may be utilized to aid in the identification of MSCs from other species, as well as to investigate/identify MSC functions in humans or other species. In a preferred aspect of the invention, immunocyto-chemistry is utilized with an antibody specific for a polypeptide according to the invention as a means for monitoring the concentration of the polypeptide according to the invention in a culture solution. The MSCs of the culture may thus be subjected to purification procedures to remove differentiated cells and help to maintain the MSCs in culture.

In accordance with yet a further aspect of the present invention, there are provided antibodies against such polypeptides and a method of employing such antibodies to detect diseases related to an overexpression or under expression of a polypeptide compressing a polypeptide with an amino acid sequence according to the present invention. Such antibodies (or active fragments) may be utilized to monitor the growth

of MSCs in a culture or to detect the location of tumors in the body.

In accordance with another aspect of the present invention there is provided a method of diagnosing a disease or a susceptibility to a disease related to a mutation in the nucleic acid sequences and the proteins encoded by such nucleic acid sequences.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptides, or polynucleotides encoding such polypeptides, for in vitro purposes related to scientific research, synthesis of DNA and manufacture of DNA vectors.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figure 1 shows a schematic of the transcript for a cDNA clone that encodes the mature slit polypeptide. The various coding regions and repeats are identified by different types of cross-hatching as shown in the figure and identified by the legend below it.

Figure 2 shows a cDNA sequence (SEQ ID. NO:1) which is that encodes the mature slit polypeptide (SEQ ID NO:2) of the present invention. Sequencing was performed using a 373 Automated DNA sequencer (Applied Biosystems, Inc.).

Figure 3 is an illustration of amino acid sequence homology between the human slit polypeptide of the present invention (labelled as hSlit) and the Drosophila slit polypeptide (labelled as dSlit). By aligning the two polypeptides in a manner that provides the essentially the largest number of aligned identical amino acids over the complete comparison area of the two sequences, and dividing the total number of identical amino acids by the total length of the comparison area (counting the individual spaces of gaps as part of the comparsion area), a 40% identity between the two amino acid sequences was observed. Standard one-letter abbreviations for amino acids are used.

Figure 4 shows a photograph of a protein blot from expression of hSlit in human embryonic kidney cell line, BOSC 23. Untransfected BOSC cells do not express hSlit. In Figure 4, Lane 1 shows the results for untransfected BOSC cells; Lanes 2, 3 and 4, respectively show (2) BOSC cells transfected with pcDNA3.1/Myc-His/A vector, (3) pcDNA3.1/Myc-His/lacZ and (4) pcDNA3.1/Myc-His/hSlit cDNA.

In accordance with an aspect of the present invention, there are provided isolated nucleic acids (polynucleotides) which encode the mature polypeptides comprises the deduced amino acid sequences of SEQ ID NO:2. The mature forms of the slit polypeptide with and without an N-terminal methionine group (N-terminal methionine is the first amino acid of SEQ ID NO:2) are contemplated.

Polynucleotides encoding the polypeptide of the present invention have been isolated from a human MSC

cDNA library. The polynucleotide contains an open reading frame encoding the human slit polypeptide. The protein exhibits a high degree of homology at the amino acid level to the Drosophila slit polypeptide with 40% identity (as shown in Figure 3).

In accordance with a further aspect of the present invention the human slit gene sequence according to SEQ ID NO:1 or an appropriate fragment (full or partial length probes) may be utilized under stringent hybridization conditions to isolate from a cDNA library prepared from MSCs by procedures known in the art the cDNA encoding alleles of the mature slit polypeptide. Further, such full- or partial- length probes may be utilized to isolate genes (or cDNAs) encoding related polypeptides from non-human hosts under either stringent or highly-stringent hybridization conditions. Likewise the polypeptide having an amino acid sequence according to SEQ ID NO:2 or an immunogenic fragment may be utilized to produce antibodies specific for the polypeptide according to SEQ ID NO:2 and a fragment thereof. Such antibodies are in turn useful to detect the presence of such polypeptides when they are expressed by a clone or a transformed host cell to indicate the presence of the respective polynucleotides encoding such polypeptides.

The polynucleotides of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptides may comprise an amino acid sequence identical to the coding sequence shown in Figure 1 (SEQ

ID NO:1) or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptides comprising the polypeptide of SEQ. ID NO:2, the cDNA for which is shown in Figure 1 (SEQ ID NO:1).

The polynucleotides which encode the mature polypeptides of the present invention comprise the polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 and may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptides.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes coding sequence for the polypeptide and may also include additional coding and/or non-coding sequence such as introns.

The present invention further relates to variants of the hereinabove described polynucleotides which encode fragments, analogs and derivatives of the mature polypeptide comprising amino acid sequence shown in SEQ ID NO:2. The variant of the polynucleotides may be a naturally occurring allelic variant of the polynucleotides or a non-naturally occurring variant of the polynucleotides.

Further particularly preferred in this regard are polynucleotides encoding the human slit polypeptide variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, which comprise the amino acid sequence of the polypeptide of SEQ ID NO:2 or of the deposit in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the human slit polypeptide. Also especially preferred in this regard are conservative substitutions. Most highly preferred are mature polypeptides comprising the amino acid sequence set forth in SEQ ID NO:2 or of the deposit, without substitutions.

Thus, the present invention includes polynucleotides encoding the same mature polypeptides comprising the polypeptide as set forth in SEQ ID NO:2 as well as variants of such polynucleotides which variants encode a fragment, derivative or analog of the polypeptides set forth in SEQ ID NO:2. Such polynucleotide variants include deletion variants, substitution variants and addition or insertion variants. Preferred are polynucleotide sequences comprising polynucleotide sequence variants of a starting polynucleotide sequence that are obtained by changing the starting polynucleotide sequence in at least one of the following ways (a) inserting at least one nucleotide into it, (b) deleting at least one nucleotide from it, (c) substituting at least one nucleotide for a nucleotide of it, or (d) a combination of at least two of (a), (b) and (c). The starting polynucleotide sequence that is changed to

obtain variant polynucleotide sequences is a member selected from (i) the coding portion of SEQ ID NO:1 and (ii) a redundant sequence encoding the same mature polypeptide as the coding portion of SEQ ID NO:1. Each of the preferred variant polynucleotide sequences results from changing no more than a total of 10 percent of the coding sequence nucleotides of the starting polynucleotide sequence by such deletion, substitution, insertion or a combination thereof (i.e., not more than 10 nucleotides per 100 nucleotides). More preferred are variant polynucleotide sequences that result from changing no more than a total of 5 percent of the starting coding sequence nucleotides by deletion, insertion, substitution or a combination thereof. Even more preferred are variant polynucleotide sequences that result from changing no more than a total of 3 percent of the starting coding sequence nucleotides by deletion, insertion, substitution or a combination thereof. Such changes occur within the 5' to 3' portions of the coding sequence of the starting polynucleotide. The polypeptides encoded by such variant polynucleotides may or may not retain the activity of the polypeptide encoded by the polynucleotide of SEQ ID NO:1. For example, such polynucleotides may be employed as probes for the gene comprising the polynucleotide of SEQ ID NO:1, for the polynucleotide of SEQ ID NO:1 or for a redundant polynucleotide which encodes the same polypeptide that is encoded by the polynucleotide having a sequence according to SEQ ID NO:1. However, preferred are the polynucleotides which encode variant polypeptides that retain substantially the same biological function or activity as the mature polypeptide comprising the amino acid sequence encoded by the cDNA of Figure 1 (SEQ ID

NO:2), or that of the amino acid sequence encoded by the polynucleotide of SEQ ID NO:1.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequences comprising the coding portion of the polynucleotide sequence shown in Figure 1 (of SEQ ID NO:1). As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The present invention also includes polynucleotides, wherein the coding sequence for the mature polypeptides may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides may also encode a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

Thus, for example, the polynucleotides of the present invention may encode a mature protein, or for a protein having a prosequence or for a protein having both a prosequence and a presequence (leader sequence).

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptides of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptides fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

Fragments of the full length gene of the present invention may be used as a hybridization probe for a cDNA library to isolate the full length cDNA and to isolate other cDNAs which have a high sequence similarity to the gene or similar biological activity. Probes of this type preferably have at least 30 bases and may contain, for example, 50 or more bases. The probe may also be used to identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete gene including regulatory and promotor regions, exons, and introns. An example of a screen comprises isolating the coding region of the gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence

complementary to that of the gene of the present invention are used to screen a library of human cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which either retain substantially the same biological function or activity as the mature polypeptide comprising the amino acid sequence encoded by the cDNA of Figure 1 (comprising SEQ ID NO:2).

Alternatively, the polynucleotide may have at least 20 bases, preferably at least 30 bases, and more preferably at least 50 bases which hybridize to a polynucleotide of the present invention and which has an identity thereto, as hereinabove described, and which may or may not retain activity. For example, such polynucleotides may be employed as probes for the gene comprising the polynucleotide of SEQ ID NO:1, for example, for recovery of the polynucleotide or as a diagnostic probe or as a PCR primer.

Thus, the present invention is directed to polynucleotides having at least a 70% identity, preferably at least 90% and more preferably at least a 95% identity to a polynucleotide which encodes the polypeptide of SEQ ID NO:2 and polynucleotides complementary thereto as well as portions thereof, which portions have at least 20, preferably at least 30 consecutive bases and may have at least 50 consecutive bases and to polypeptides encoded by such polynucleotides.

The present invention further relates to polypeptides which have the deduced amino acid sequence as set forth in SEQ ID NO:2, as well as fragments, analogs and derivatives of such polypeptides.

The terms "fragment," "derivative" and "analog" when referring to the mature polypeptides comprising the polypeptide as set forth in SEQ ID NO:2, means polypeptides which retain essentially the same biological function or activity as such polypeptides. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

Among the particularly preferred embodiments of the invention in this regard are mature polypeptides comprising the amino acid sequence as set forth in SEQ ID NO:2, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments. Alternatively, particularly preferred embodiments of the invention in this regard are polypeptides comprising the amino acid sequence of the human slit polypeptide encoded by the cDNA in the

deposited clone, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments.

Further particularly preferred in this regard are variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, comprising the amino acid sequence of the polypeptide as set forth in SEQ ID NO:2 or as encoded by the cDNA in the deposited clone, in which at least one amino acid residue per each 100 amino acids of the amino sequence is varied by at least one of (a) substituting an amino acid for it, (b) deleting at least one amino acid, (c) inserting at least one new amino acid, or (d) a combination of at least two of (a), (b) and (c). For example, variant polypeptides are obtained whose amino acid sequences are obtained by changing 5 to 10, 1 to 5, 1 to 3, or 1 to 2 amino acid residues per 100 amino acids in that at least one of (i) at least one new amino acid is substituted for an amino acid of SEQ ID NO:2 (or of a fragment of SEQ ID NO:2), (ii) at least one amino acid of SEQ ID NO:2 (or of a fragment of SEQ ID NO:2) is deleted, (ii) at least one new amino acid is inserted into SEQ ID NO:2 (or into a fragment of SEQ ID NO:2), or (iv) a combination of (i), (ii) or (iii). Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of such polypeptide as compared to those properties and activities of the human slit polypeptide. Also especially preferred in this regard are conservative substitutions. Most highly preferred are mature polypeptides comprising the amino acid sequence as set forth in SEQ ID NO:2, or of the deposited clone, without substitutions.

The polypeptides of the present invention may be recombinant polypeptides, natural polypeptides or synthetic polypeptides, preferably recombinant polypeptides.

The fragment, derivative or analog of the polypeptides comprising the amino acid sequence set forth in SEQ ID NO:2 may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide

present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The polypeptides of the present invention include polypeptides comprising the polypeptide of SEQ ID NO:2 (in particular the mature polypeptide) as well as polypeptides which have at least 70% similarity (preferably at least 70% identity) to the mature polypeptide comprising the amino acid sequence of SEQ ID NO:2, and which have at least 90% similarity (more preferably at least 90% identity) to the mature polypeptide comprising the amino acid sequence of SEQ ID NO:2 and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the mature polypeptide comprising the amino acid sequence of SEQ ID NO:2 and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

As known in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. For such a determination, two amino acid sequences are compared along a stretch of their sequences, any gap (or gaps) introduced in one sequence to improve the alignment and similarity to the other sequences is counted as

spaces of dissimilarity equal to the number of amino acids corresponding to the gap which are present in the second sequence, and the total number of similar amino acids are divided by the total number of amino acids present in the comparison area which counts the spaces of gaps as part of the comparsion area.

Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length polypeptides. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda PL promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example.

Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pBS, pD10, phagescript, psiX174, pBluescript SK, pBSKS, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); pTRC99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene products encoded by the recombinant sequences. Alternatively, the

polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock

proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA).

These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa, 293 and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and

polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The polypeptides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics for human disease. For example, the polynucleotides and polypeptides encoded by such polynucleotides may also be utilized for *in vitro* purposes related to scientific research, synthesis of DNA and manufacture of DNA vectors

and for designing therapeutics and diagnostics for human disease.

The invention also provides a method for identifying human mesenchymal stem cells by contacting a mixture of mRNA from a cell sample with a polynucleotide unique to human *slit* and identifying any mRNA which has hybridized with the polynucleotide unique to human *slit*. In a preferred embodiment the polynucleotide unique to human *slit* is bound to a solid support. Thus, for example, the identification of *slit* cDNA enables the *slit* nucleic acid sequence to be utilized as a diagnostic reagent to identify human MSCs, such as by using gene expression array technology. Labeled (*e.g.* fluorescent or radiolabeled) mixtures of total cellular mRNA hybridize to cognate elements of *slit* on a chip based array and allow for the accurate detection of genes specific to MSCs. This technology is described, for example, in Schena, *Bioessays, 18(5)*:427-431 (May 1996) and O'Donnell-Maloney & Little, *Genet. Anal., 13(6)*:151-157 (Dec. 1996).

The polypeptides of the present invention and fragments and analogs and derivatives thereof may be identified by assays which detect MSC proliferation or other activity. Further, assays may be utilized which neutralize the production of the native *slit* in midline glia cells and subjecting such cells to a polypeptide sequence which is related to the native *slit* sequence but is different in order to verify the same functionality of polypeptides having both sequences.

This invention is also related to the use of the human *slit* polypeptide gene as part of a diagnostic assay

for detecting diseases or susceptibility to diseases related to the presence of mutations in the human *slit* polypeptide nucleic acid sequences. Such diseases are related to under-expression or overexpression of the human *slit* polypeptides.

Individuals carrying mutations in the human *slit* polypeptide gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki *et al.*, Nature, 324:163-166 (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid encoding human *slit* polypeptide can be used to identify and analyze human *slit* polypeptide mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled human *slit* polypeptide RNA or alternatively, radiolabeled human *slit* polypeptide antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences

may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers *et al.*, Science, 230:1242 (1985)).

Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton *et al.*, PNAS, USA, 85:4397-4401 (1985)).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., Restriction Fragment Length Polymorphisms (RFLP)) and Southern blotting of genomic DNA.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

The present invention also relates to a diagnostic assay for detecting altered levels of the *slit* polypeptide in various tissues since an over-expression or under-expression of the proteins compared to normal control tissue samples may detect the presence of a disease or susceptibility to a disease, for example, reduced blood cell counts or malignancies such as cancers and tumors. Assays used to detect levels of the *slit* polypeptide in a sample derived from a host are well-known to those of skill in the art and include radioimmunoassays, competitive-binding assays, Western Blot analysis, ELISA assays and "sandwich" assay. An

ELISA assay (Coligan, et al., Current Protocols in Immunology, 1(2), Chapter 6, (1991)) initially comprises preparing an antibody specific to the *slit* polypeptide antigen, preferably a monoclonal antibody. In addition a reporter antibody is prepared against the monoclonal antibody. To the reporter antibody is attached a detectable reagent such as radioactivity, fluorescence or, in this example, a horseradish peroxidase enzyme. A sample is removed from a host and incubated on a solid support, e.g. a polystyrene dish, that binds the proteins in the sample. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein like BSA. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any *slit* polypeptide attached to the polystyrene dish. All unbound monoclonal antibody is washed out with buffer. The reporter antibody linked to horseradish peroxidase is now placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to the *slit* polypeptide. Unattached reporter antibody is then washed out. Peroxidase substrates are then added to the dish and the amount of color developed in a given time period is a measurement of the amount of the *slit* polypeptide present in a given volume of patient sample when compared against a standard curve.

A competition assay may be employed wherein antibodies specific to the *slit* polypeptide are attached to a solid support and labeled the *slit* polypeptide and a sample derived from the host are passed over the solid support and the amount of label detected, for example by liquid scintillation chromatography, can be correlated to a quantity of the *slit* polypeptide in the sample.

A "sandwich" assay is similar to an ELISA assay. In a "sandwich" assay the *slit* polypeptide is passed over a solid support and binds to antibody attached to a solid support. A second antibody is then bound to the *slit* polypeptide. A third antibody which is labeled and specific to the second antibody is then passed over the solid support and binds to the second antibody and an amount can then be quantified.

This invention provides a method for identification of the receptors for the human *slit* polypeptides. The gene encoding the receptor can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the labeled polypeptides. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for the *slit* polypeptides. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and retransfected using an iterative sub-pooling and rescreening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be

photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

This invention provides a method of screening compounds to identify agonists and antagonists to the human *slit* polypeptides of the present invention. An agonist is a compound which has similar biological functions of the polypeptides, while antagonists block such functions. Antagonists and agonists may be identified by the an MSC proliferation assay as is well known in the art.

Examples of potential the *slit* polypeptide antagonists include antibodies, or in some cases, oligonucleotides, which bind to the polypeptides. Another example of a potential antagonist is a negative dominant mutant of the polypeptides. Negative dominant mutants are polypeptides which bind to the receptor of the wild-type polypeptide, but fail to retain biological activity.

Antisense constructs prepared using antisense technology are also potential antagonists. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of

which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple- helix, see Lee et al., Nucl. Acids Res., 6:3073 (1979); Cooney et al, Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991)), thereby preventing transcription and the production of the human *slit* polypeptide. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the polypeptides (antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the human *slit* polypeptide.

Another potential human *slit* antagonist is a peptide derivative of the polypeptides which are naturally or synthetically modified analogs of the polypeptides that have lost biological function yet still recognize and bind to the receptors of the polypeptides to thereby effectively block the receptors. Examples of peptide derivatives include, but are not limited to, small peptides or peptide-like molecules.

The antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

The human *slit* polypeptides and antagonists may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the polypeptide, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides and agonists and antagonists may be employed in conjunction with other therapeutic compounds.

The pharmaceutical compositions may be administered in a convenient manner such as by the topical, intravenous, intraperitoneal, intramuscular, intratumor, subcutaneous, intranasal or intradermal routes. The pharmaceutical compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, the polypeptides will be administered in an amount of at least about 10 μg/kg body weight and in most cases they will be administered in an amount not in excess of about 8 mg/Kg body weight per day. In most cases, the dosage is from about 10

μg/kg to about 1 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc.

The human *slit* polypeptides, and agonists or antagonists which are polypeptides, may be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by, for example, procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells in vivo after combination with a suitable delivery vehicle.

Retroviruses from which the retroviral plasmid vectors hereinabove mentioned may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus. In one embodiment, the retroviral plasmid vector is derived from Moloney Murine Leukemia Virus.

The vector includes one or more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, et al., Biotechniques, Vol. 7, No. 9, 980-990 (1989), or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The nucleic acid sequence encoding the polypeptide of the present invention is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the

albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs hereinabove described); the β-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter which controls the gene encoding the polypeptide.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, ι-2, ι-AM, PA12, T19-14X, VT-19-17-H2, ιCRE, ιCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy, Vol. 1, pgs. 5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma

cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells. The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for

PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clones to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60 bases. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then

identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner,

even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

Antibodies specific to the polypeptide of the present invention may be employed as a diagnostic to determine elevated or lowered levels of the polypeptide in a sample derived from a host by techniques known in the art. These elevated or lowered levels are indicative of certain disorders which are characterized by such levels of the protein of the present invention and members of its family.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

Such antibodies to the polypeptides of the present invention may be utilized to detect the presence or the absence of the polypeptides of the present invention. Thus, they are useful in an assay to verify the successful insertion of the polynucleotides of the present invention (as part of a construct) into a host cell. Thus, the protein encoded by the inserted polynucleotide according to the present invention, when expressed by the transformed host cell, serves as a "marker" for the successful insertion of the polynucleotide that can be detected by an antibody for the marker.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume.

Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

"Identity" means, as utilized in the context of the present specification and claims, a homology comparison with respect to the degree of sameness between a first sequence and a second sequence (the first sequence may

also be referred to as the "reference sequence"). Identity is expressed as the ratio N/D times 100 percent, where N is the number of identical aligned items (bases or amino acids) and D is the sum of the total number of items in the reference sequence and the total individual spaces (corresponding to items in the second sequence) introduced into the reference sequence as a result of its alignment with the second sequence. Further, the alignment by which the N/D ratio of identity is obtained is an alignment which gives essentially the largest possible percentage identity value, *i.e.*, the largest N value (the largest number of aligned sequence items that are identical) and the smallest D value (the smallest number of individual gap spaces introduced into the reference sequence by the alignment). Ascertaining absolutely the highest possible identity value (or best alignment) is not required to report an "essentially largest identity value" since this means in the context of the present application that the percentage identity reported has a certainty deviation that limits any possible increases in the identity value due to an alternative alignment to less than one-half of a percentage point. The sequence alignment utilized to obtain the N/D percentage identity may be performed by a manual method (hand and eye alignment) or by utilizing commercially available alignment software. The parameters of the alignment software may be adjusted until an identity value is obtained which has a certainty that limits any increase in the identity value to less than one-half of a percentage point with respect to the reported identity value.

"At Least X Percent Identity" means, as used in the context of the present specification or claims, a

homology comparison with respect to the degree of sameness between a first sequence and a second sequence (the first sequence may also be referred to as the "reference sequence") wherein the degree of sameness is equal to or exceeds the value "X" of the term. The "identity" value (degree of sameness) of this term is expressed as the ratio N/D times 100 percent, where N is the number of identical aligned items (bases or amino acids) and D is the sum of the total number of items in the reference sequence and the total individual spaces (corresponding to items in the second sequence) introduced into the reference sequence as a result of its alignment with the second sequence. If any alignment exists for the second sequence and the reference sequence which results in a sameness value (N/D x 100%) that is equal to or greater than the value of "X" in the phrase "at least X percent identity" then the second sequence has "at least X percent identity" with respect to the reference sequence even though it may be possible to align the two sequence in a different manner such that the calculated value is less than X. The sequence alignment utilized to obtain the N/D percentage identity may be performed by a manual method (hand and eye alignment) or by utilizing commercially available alignment software, provided that the "identity" value is calculated as hereinabove described.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to

such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the invention the following examples providing certain frequently occurring methods and/or terms will be described.

Example 1

PCR Amplification of Human *Slit*

The cDNA sequence coding for human *slit* is obtained from a cDNA library containing it (such as from MSCs or stem cells) and amplified by PCR using the oligonucleotide primers corresponding to the 5' and 3' end sequences of the processed *slit* nucleic acid sequence. Additional nucleotides corresponding to the *slit* gene are added to the 5' and 3' end sequences of the processed *slit* nucleic acid sequence.

For example, the following PCR primers may be utilized for amplification of the cDNA:

5' primer = TCCTCGGGCTCCACGCGTCTT (SEQ ID NO:3), and

3' primer = GGTACATATACGCAGATGGTG (SEQ ID NO:4).

Standard PCR amplification kits are available in the art and may be utilized for such amplification by following the PCR amplification instructions provided therewith.

Isolation of the full-length cDNA may be done utilizing methods standard in the art.

Furthermore, the amplified cDNA may be utilized to produce the polypeptide which it encodes by utilizing methods standard in the art.

Example 2

Expression and Purification of Human *Slit*

The cDNA sequence coding for human *slit* is obtained from a cDNA library and may be amplified as set forth in Example 1, above.

A. Construction of expression plasmid

The full-length hslit cDNA fragment encompassing an EcoR1 site at the 5'-end and engineered to contain a Kpn1 site just before the termination codon was cloned into EcoR1, Kpn1 digested mammalian expression vector, pcDNA3,1/Myc-His/A (Invitrogen, Carlsbad, CA) such that the open reading frame of hSlit cDNA was in phase with the C-terminal myc epitope and the polyhistidine tag.

The pcDNA 3.1 vector was utilized in that it is designed for high level expression and purification of recombinant proteins in mammalian cells. The human cytomegalovirus (CMV) promoter was utilized to provide high level expression in a wide range of mammalian cells. The myc epitope and the his tag utilized allow tracking and purification of the expressed protein using commercially available (Invitrogen, Carlsbad, CA) anti-myc antibodies and metal-chelating resin, respectively.

B. Transfection of BOSC 23 cells

The human embryonic kidney cell line, BOSC 23, which does not express hSlit, and which can be transfected at a very high efficiency, was used for expression of hSlit.

BOSC 23 cells were transiently transfected with vector, or control plasmid, pcDNA3.1/Myc-His/lacZ, or pcDNA3.1/Myc-His/hSlit DNA using the standard calcium phosphate precipitation method. Forty-eight hours post-transfection, total cell lysates were prepared from the transfected and untransfected control cells and analyzed by Western blotting.

C. Western Analysis

Protein content of the cell lysates was estimated using the BCA reagent (Pierce, Rockford, IL). Approximately 100 µg of protein from various samples was electrophoresed on a 7.5% SDS-PAGE gel, and electrophoretically transferred to Immobilon PVDF membrane (Milipore, Bedford, MA). The protein blot was probed with anti-myc antibodies using ECL detection reagents and protocol (Amersham, UK).

Such procedures are standard in the art. Briefly, the blot was incubated with 5% milk to block non-specific binding sites, followed by incubation with a 1:5000 dilution of anti-myc mouse monoclonal antibodies, and finally incubation with a 1:3000 dilution of anti-mouse Ig linked to horse radish peroxidase. The antibody binding was detected using ECL detection reagents and exposure to X-ray film.

C. Western Analysis Results

The results are shown in the Figure 4. In Figure 4, Lane 1 shows the results for untransfected BOSC cells; Lanes 2, 3 and 4, respectively, show (2) BOSC cells transfected with pcDNA3.1/Myc-His/A vector, (3) pcDNA3.1/Myc-His/lacZ and (4) pcDNA3.1/Myc-His/hSlit cDNA.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims. Further, the invention may be readily adapted and practiced otherwise than as particularly described.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising a polynucleotide sequence which is a member selected from the group consisting of:

(a) a polynucleotide encoding amino acid 2 to 1523 of SEQ ID NO:2;

(b) a variant polynucleotide sequence of (a), wherein said variant polynucleotide sequence varies from the polynucleotide sequence of (a) by a member selected from (i) nucleotide substitution, (ii) nucleotide deletion, (iii) nucleotide insertion, and (iv) a combination of (i), (ii) or (iii), and said variant polynucleotide will hybridize to the complement of a polynucleotide of (a),

(c) the full complement of (a); and

(d) the full complement of (b).

2. An isolated polynucleotide comprising a polynucleotide having at least 95% identity to a member selected from the group consisting of:

(a) a polynucleotide encoding amino acid 2 to 1523 of SEQ ID NO:2; and

(b) the full complement of (a).

3. The isolated polynucleotide of claim 1 wherein said member is (a) or (b).

4. The isolated polynucleotide of claim 1 comprising a polynucleotide encoding a polypeptide comprising amino acids 2 to 1523 of SEQ ID NO:2.

5. The isolated polynucleotide of claim 1, wherein said member is (a) or (b) and the polynucleotide is DNA.

6. A recombinant vector comprising the polynucleotide of claim 3, wherein said polynucleotide is DNA.

7. A recombinant host cell comprising the polynucleotide of claim 3, wherein said polynucleotide is DNA.

8. A method for producing a polypeptide comprising expressing from the recombinant cell of claim 7 the polypeptide encoded by said polynucleotide.

9. A process for producing a mature *slit* polypeptide comprising:

expressing from a recombinant cell containing the polynucleotide of claim 4 the polypeptide encoded by said polynucleotide.

10. The isolated polynucleotide of claim 1 comprising the nucleotides of the sequence of SEQ ID NO:1.

11. An isolated polypeptide comprising:

a mature polypeptide having an amino acid sequence encoded by a polynucleotide which is at least 95% identical to the polynucleotide of claim 4.

12. The isolated polypeptide of claim 11, comprising amino acids 1 to 1523 of sequence of SEQ ID NO:2.

13. An antibody against the polypeptide of claim 11.

14. An antagonist against the polypeptide of claim 11.

15. A method for the treatment of a patient having need of a human *slit* polypeptide comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 11.

16. The method of Claim 15 wherein said therapeutically effective amount of the polypeptide is administered by providing to the patient DNA encoding said polypeptide and expressing said polypeptide *in vivo*.

17. A method for the treatment of a patient having need to inhibit the activity of a human *slit* polypeptide comprising: administering to the patient a therapeutically effective amount of the antagonist of Claim 14.

18. A method for the treatment of a patient having need of a human *slit* polypeptide comprising: administering to the patient a therapeutically effective amount of the agonist of Claim 15.

19. A process for diagnosing a disease or a susceptibility to a disease related to expression of the polypeptide of claim 11 comprising:

determining a mutation in the nucleic acid sequence encoding said polypeptide.

20. A diagnostic process comprising:

analyzing for the presence of the polypeptide of claim 11 in a sample derived from a host.

FIG. 1

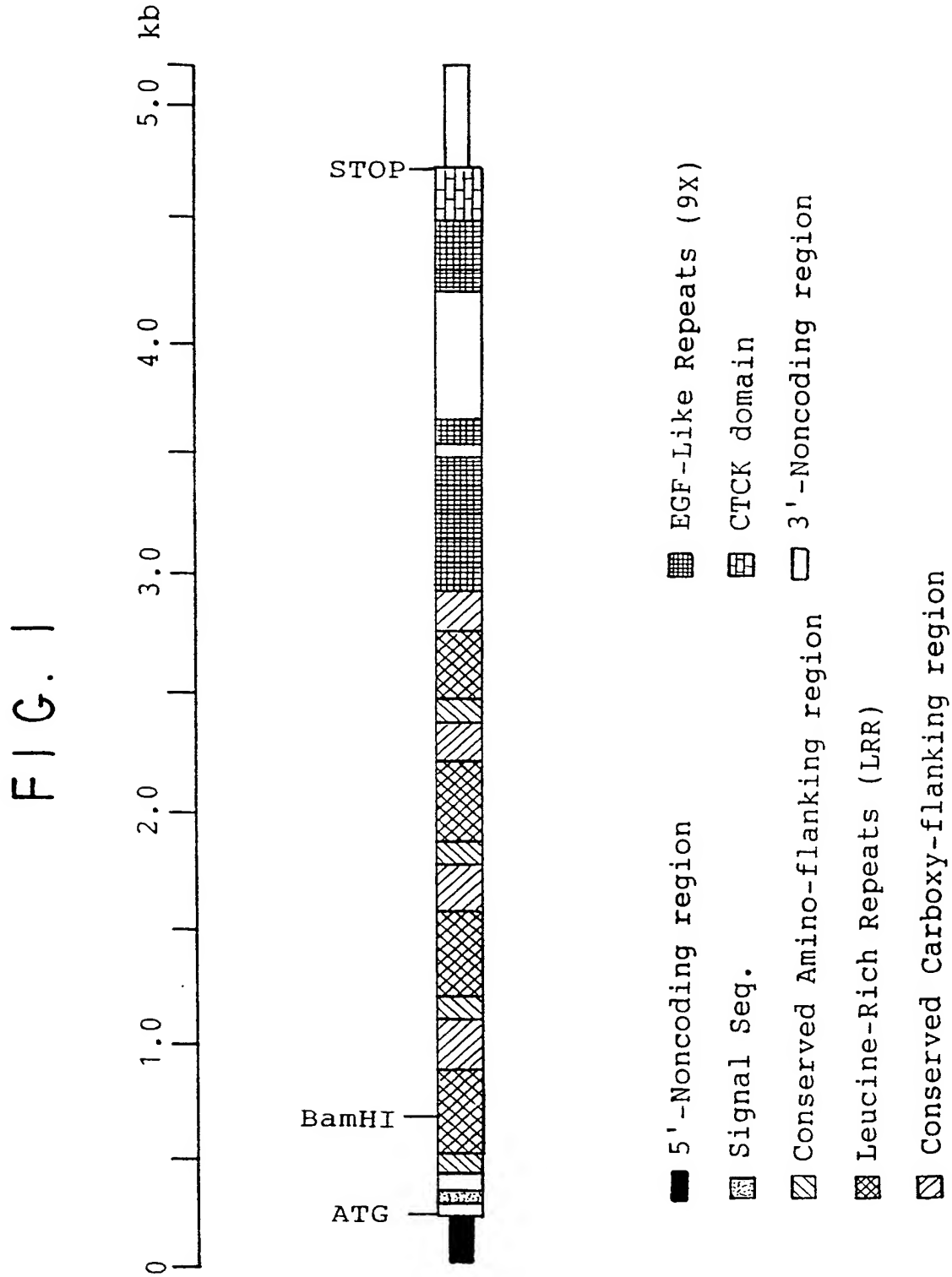


FIG. 2

FIG. 2A	FIG. 2B
FIG. 2C	FIG. 2D
FIG. 2E	FIG. 2F
FIG. 2G	FIG. 2H
FIG. 2I	FIG. 2J
FIG. 2K	FIG. 2L
FIG. 2M	FIG. 2N

FIG. 2A

```
1                                                GCCGGCCCCGCCGATGGAGCT
62          GCCCGCGCCCCGTGCGCCTGAGCACCGAGCTCGCCCCTC
141         GCTCCCCGCGCGCCTCCTCGGGCTCCACGCGTCTTGCCC

220         ATG GCC CCC GGG TGG GCA GGG GTC GGC GCC
1           Met ala pro gly trp ala gly val gly ala

280         GCG CTG GCG AGC GTC CTG AGT GGG CCT CCA
21          ala leu ala ser val leu ser gly pro pro

340         TCC GCT GCC AGC GTG GAC TGC CAC GGG CTG
41          ser ala ala ser val asp cys his gly leu

400         CGC AAC GCT GAG CGC CTT GAC CTG GAC AGA
61          arg asn ala glu arg leu asp leu asp arg

460         TTC GCT GGG CTC AAG AAC CTC CGA GTC TTG
81          phe ala gly leu lys asn leu arg val leu

520         GAG AGA GGC GCC TTC CAG GAC CTG AAG CAG
101         glu arg gly ala phe gln asp leu lys gln

580         CTG CAA GTC CTT CCA GAA TTG CTT TTC CAG
121         leu gln val leu pro glu leu leu phe gln

640         AGT GAA AAC CAG ATC CAG GGG ATC CCG AGG
141         ser glu asn gln ile gln gly ile pro arg

700         AAC CTG CAA CTG GAC AAC AAC CAC ATC AGC
161         asn leu gln leu asp asn asn his ile ser

760         CGC GAT TTG GAG ATC CTT ACC CTC AAC AAC
181         arg asp leu glu ile leu thr leu asn asn

820         TTC AAC CAC ATG CCG AAG ATC CGA ACT CTG
201         phe asn his met pro lys ile arg thr leu
```

MATCH WITH FIG. 2C

MATCH WITH FIG. 2B

FIG. 2B

GCTGTTGCTGCCGCCGCCGCCTCCCGGAGCGCCCCGCTCC
CTCCGCGCTAACTCCGCCGCCCGCTCCCCAGGCCGCCCGC
CGCAGAGGCAGCCTCCTCCAGGAGCGGGGCCCTGCACACC

GCC GTG CGC GCC CGC CTG GCG CTG GCC TTG
ala val arg ala arg *leu ala leu ala leu*

GCC GTC GCC TGC CCC ACC AAG TGT ACC TGC
ala val ala/cys pro thr lys cys thr cys

GGC CTC CGC GCG GTT CCT CGG GGC ATC CCC
gly leu arg ala val pro arg gly ile pro

AAT AAT ATC ACC AGG ATC ACC AAG ATG GAC
asn asn ile thr arg ile thr lys met asp

CAT CTG GAA GAC AAC CAG GTC AGC GTC ATC
his leu glu asp asn gln val ser val ile

CTA GAG CGA CTG CGC CTG AAC AAG AAT AAG
leu glu arg leu arg leu asn lys asn lys

AGC ACG CCG AAG CTC ACC AGA CTA GAT TTG
ser thr pro lys leu thr arg leu asp leu

AAG GCG TTC CGC GGC ATC ACC GAT GTG AAG
lys ala phe arg gly ile thr asp val lys

TGC ATT GAA GAT GGA GCC TTC CGA GCG CTG
cys ile glu asp gly ala phe arg ala leu

AAC AAC ATC AGT CGC ATC CTG GTC ACC AGC
asn asn ile ser arg ile leu val thr ser

CGC CTC CAC TCC AAC CAC CTG TAC TGC GAC
arg leu his ser asn his leu tyr cys asp

MATCH WITH FIG. 2A

MATCH WITH FIG. 2D

MATCH WITH FIG. 2A

FIG. 2C

```
880     TGC CAC CTG GCC TGG CTC TCG GAT TGG CTG
221     cys his leu ala trp leu ser asp trp leu

940     CTC TGC ATG GCT CCT GTG CAT TTG AGG GGC
241     leu cys met ala pro val his leu arg gly

1000    TAC GTG TGC CCA GCC CCC CAC TCG GAG CCC
261     tyr val cys pro ala pro his ser glu pro

1060    CCT TCG CCC TGC ACG TGC AGC AAT AAC ATC
281     pro ser pro cys thr cys ser asn asn ile

1120    ATT CCT GCC AAT TTG CCG GAG GGC ATC GTC
301     ile pro ala asn leu pro glu gly ile val

1180    GCC ATC CCT GCA GGA GCC TTC ACC CAG TAC
321     ala ile pro ala gly ala phe thr gln tyr

1240    AAT CAG ATA TCG GAT ATT GCT CCA GAT GCC
341     asn gln ile ser asp ile ala pro asp ala

1300    GTC CTG TAT GGG AAC AAG ATC ACC GAG ATT
361     val leu tyr gly asn lys ile thr glu ile

1360    CTA CAG CTG CTC CTC CTC AAT GCC AAC AAG
381     leu gln leu leu leu leu asn ala asn lys

1420    GAC CTG CAG AAC CTC AAC TTG CTC TCC CTG
401     asp leu gln asn leu asn leu leu ser leu

1480    GGG CTC TTC GCC CCT CTG CAG TCC ATC CAG
421     gly leu phe ala pro leu gln ser ile gln

1540    TGC GAC TGC CAC TTG AAG TGG CTG GCC GAC
441     cys asp cys his leu lys trp leu ala asp
```

MATCH WITH FIG. 2D

MATCH WITH FIG. 2E

FIG. 2D 6/22 MATCH WITH FIG. 2B

CGA CAG CGA CGG ACA GTT GGC CAG TTC ACA
arg gln arg arg thr val gly gln phe thr

TTC AAC GTG GCG GAT GTG CAG AAG AAG GAG
phe asn val ala asp val gln lys lys glu

CCA TCC TGC AAT GCC AAC TCC ATC TCC TGC
pro ser cys asn ala asn ser ile ser cys

GTG GAC TGT CGA GGA AAG GGC TTG ATG GAG
val asp cys arg gly lys gly leu met glu

GAA ATA CGC CTA GAA CAG AAC TCC ATC AAA
glu ile arg leu glu gln asn ser ile lys

MATCH WITH FIG. 2C

AAG AAA CTG AAG CGA ATA GAC ATC AGC AAG
lys lys leu lys arg ile asp ile ser lys

TTC CAG GGC CTG AAA TCA CTC ACA TCG CTG
phe gln gly leu lys ser leu thr ser leu

GCC AAG GGA CTG TTT GAT GGG CTG GTG TCC
ala lys gly leu phe asp gly leu val ser

ATC AAC TGC CTG CGG GTG AAC ACG TTT CAG
ile asn cys leu arg val asn thr phe gln

TAT GAC AAC AAG CTG CAG ACC ATC AGC AAG
tyr asp asn lys leu gln thr ile ser lys

ACA CTC CAC TTA GCC CAA AAC CCA TTT GTG
thr leu his leu ala gln asn pro phe val

TAC CTC CAG GAC AAC CCC ATC GAG ACA AGC
tyr leu gln asp asn pro ile glu thr ser

MATCH With FIG. 2F

MATCH WITH FIG. 2C

FIG. 2E

```
1600  GGG GCC CGC TGC AGC AGC CCG CGC CGA CTC
461   gly ala arg cys ser ser pro arg arg leu

1660  AAG AAG TTC CGC TGC TCA GGC TCC GAG GAT
481   lys lys phe arg cys ser gly ser glu asp

1720  ATG GAC CTC GTG TGC CCC GAG AAG TGT CGC
501   met asp leu val cys pro glu lys cys arg

1780  CAG AAG CTG GTC CGC ATC CCA AGC CAC CTC
521   gln lys leu val arg ile pro ser his leu

1840  GAC AAT GAG GTA TCT GTT CTG GAG GCC ACT
541   asp asn glu val ser val leu glu ala thr

1900  AAA ATA AAT CTG AGT AAC AAT AAG ATC AAG
561   lys ile asn leu ser asn asn lys ile lys

1960  GCC AGC GTG CAG GAG CTG ATG CTG ACA GGG
581   ala ser val gln glu leu met leu thr gly

2020  TTC CGT GGC CTC AGT GGC CTC AAA ACC TTG
601   phe arg gly leu ser gly leu lys thr leu

2080  AGT AAT GAC ACC TTT GCC GGC CTG AGT TCG
621   ser asn asp thr phe ala gly leu ser ser

2140  ATC ACC ACC ATC ACC CCT GGG GCC TTC ACC
641   ile thr thr ile thr pro gly ala phe thr

2200  CTG TCC AAC CCC TTC AAC TGC AAC TGC CAC
661   leu ser asn pro phe asn cys asn cys his

2260  AGG CGG ATC GTC AGT GGG AAC CCT AGG TGC
681   arg arg ile val ser gly asn pro arg cys
```

MATCH WITH FIG. 2F

MATCH WITH FIG. 2G

MATCH WITH FIG. 2D

FIG. 2F

GCC AAC AAG CGC ATC AGC CAG ATC AAG AGC
ala asn lys arg ile ser gln ile lys ser

TAC CGC AGC AGG TTC AGC AGC GAG TGC TTC
tyr arg ser arg phe ser ser glu cys phe

TGT GAG GGC ACG ATT GTG GAC TGC TCC AAC
cys glu gly thr ile val asp cys ser asn

CCT GAA TAT GTC ACC GAC CTG CGA CTG AAT
pro glu tyr val thr asp leu arg leu asn

GGC ATC TTC AAG AAG TTG CCC AAC CTG CGG
gly ile phe lys lys leu pro asn leu arg

MATCH WITH FIG. 2E

GAG GTG CGA GAG GGA GCT TTC GAT GGA GCA
glu val arg glu gly ala phe asp gly ala

AAC CAG CTG GAG ACC GTG CAC GGG CGC GTG
asn gln leu glu thr val his gly arg val

ATG CTG AGG AGT AAC TTG ATC AGC TGT GTG
met leu arg ser asn leu ile ser cys val

GTG AGA CTG CTG TCC CTC TAT GAC AAT CGG
val arg leu leu ser leu tyr asp asn arg

ACG CTT GTC TCC CTG TCC ACC ATA AAC CTC
thr leu val ser leu ser thr ile asn leu

CTG GCC TGG CTC GGC AAG TGG TTG AGG AAG
leu ala trp leu gly lys trp leu arg lys

CAG AAG CCA TTT TTC CTC AAG GAG ATC CCC
gln lys pro phe phe leu lys glu ile pro

MATCH WITH FIG. 2H

MATCH WITH FIG. 2E

FIG. 2G

```
2320   ATC CAG GAT GTG GCC ATC CAG GAC TTC ACC
701    ile gln asp val ala ile gln asp phe thr

2380   CTG AGC CCG CGC TGC CCG GAG CAG TGC ACC
721    leu ser pro arg cys pro glu gln cys thr

2440   AAG GGG CTC CGC GCC CTC CCC AGA GGC ATG
741    lys gly leu arg ala leu pro arg gly met

2500   GGA AAC CAC CTA ACA GCC GTG CCC AGA GAG
761    gly asn his leu thr ala val pro arg glu

2560   GAC CTG AGC AAC AAC AGC ATC AGC ATG CTG
781    asp leu ser asn asn ser ile ser met leu

2620   CTC TCC ACT CTG ATC CTG AGC TAC AAC CGG
801    leu ser thr leu ile leu ser tyr asn arg

2680   GGG CTG CGG TCC CTG CGA GTG CTA ACC CTC
821    gly leu arg ser leu arg val leu thr leu

2740   GGC TCC TTC AAC GAC CTC ACA TCT CTT TCC
841    gly ser phe asn asp leu thr ser leu ser

2800   TGT GAC TGC AGT CTT CGG TGG CTG TCG GAG
861    cys asp cys ser leu arg trp leu ser glu

2860   ATC GCC CGC TGC AGT AGC CCT GAG CCC ATG
881    ile ala arg cys ser ser pro glu pro met

2920   CAC CGC TTC CAG TGC AAA GGG CCA GTG GAC
901    his arg phe gln cys lys gly pro val asp

2980   CTC TCC AGC CCG TGC AAG AAT AAC GGG ACA
921    leu ser ser pro cys lys asn asn gly thr
```

MATCH WITH FIG. 2H

MATCH WITH FIG. 2I

MATCH WITH FIG. 2F

FIG. 2H

```
TGT GAT GGC AAC GAG GAG AGT AGC TGC CAG
cys asp gly asn glu glu ser ser cys gln

TGT ATG GAG ACA GTG GTG CGA TGC AGC AAC
cys met glu thr val val arg cys ser asn

CCC AAG GAT GTG ACC GAG CTG TAC CTG GAA
pro lys asp val thr glu leu tyr leu glu

CTG TCC GCC CTC CGA CAC CTG ACG CTT ATT
leu ser ala leu arg his leu thr leu ile

ACC AAT TAC ACC TTC AGT AAC ATG TCT CAC
thr asn tyr thr phe ser asn met ser his

CTG AGG TGC ATC CCC GTC CAC GCC TTC AAC
leu arg cys ile pro val his ala phe asn

CAT GGC AAT GAC ATT TCC AGC GTT CCT GAA
his gly asn asp ile ser ser val pro glu

CAT CTG GCG CTG GGA ACC AAC CCA CTC CAC
his leu ala leu gly thr asn pro leu his

TGG GTG AAG GCG GGG TAC AAG GAG CCT GGC
trp val lys ala gly tyr lys glu pro gly

GCT GAC AGG CTC CTG CTC ACC ACC CCA ACC
ala asp arg leu leu leu thr thr pro thr

ATC AAC ATT GTG GCC AAA TGC AAT GCC TGC
ile asn ile val ala lys cys asn ala cys

TGC ACC CAG GAC CCT GTG GAG CTG TAC CGC
cys thr gln asp pro val glu leu tyr arg
```

MATCH WITH FIG. 2G

MATCH WITH FIG. 2J

FIG. 2I

MATCH WITH FIG. 2G

```
3040   TGT GCC TGC CCC TAC AGC TAC AAG GGC AAG
941    cys ala cys pro tyr ser tyr lys gly lys

3100   CAG AAC CCC TGT CAG CAT GGA GGC ACC TGC
961    gln asn pro cys gln his gly gly thr cys

3160   AGC TGC TCC TGC CCT CTG GGC TTT GAG GGG
981    ser cys ser cys pro leu gly phe glu gly

3220   GAG GAC AAC GAC TGC GAA AAC AAT GCC ACC
1001   glu asp asn asp cys glu asn asn ala thr

3280   ATC TGT CCG CCT AAC TAC ACA GGT GAG CTA
1021   ile cys pro pro asn tyr thr gly glu leu

3340   GAG CTG AAC CTC TGT CAG CAT GAG GCC AAG
1041   glu leu asn leu cys gln his glu ala lys

3400   GAG TGT GTC CCT GGC TAC AGC GGG AAG CTC
1061   glu cys val pro gly tyr ser gly lys leu

3460   CAC AAG TGC CGC CAC GGG GCC CAG TGC GTG
1081   his lys cys arg his gly ala gln cys val

3520   CCC CAG GGC TTC AGT GGA CCC TTC TGT GAA
1101   pro gln gly phe ser gly pro phe cys glu

3580   AGC CCA TGC GAC CAG TAC GAG TGC CAG AAC
1121   ser pro cys asp gln tyr glu cys gln asn

3640   CCC ACC TGC CGC TGC CCA CCA GGC TTC GCC
1141   pro thr cys arg cys pro pro gly phe ala

3700   AAC TTC GTG GGC AAA GAC TCC TAC GTG GAA
1161   asn phe val gly lys asp ser tyr val glu
```

MATCH WITH FIG. 2K

MATCH WITH FIG. 2J

FIG. 2J

MATCH WITH FIG. 2H

GAC TGC ACT GTG CCC ATC AAC ACC TGC ATC
asp cys thr val pro ile asn thr cys ile

CAC CTG AGT GAC AGC CAC AAG GAT GGG TTC
his leu ser asp ser his lys asp gly phe

CAG CGG TGT GAG ATC AAC CCA GAT GAC TGT
gln arg cys glu ile asn pro asp asp cys

TGC GTG GAC GGG ATC AAC AAC TAC GTG TGT
cys val asp gly ile asn asn tyr val cys

TGC GAC GAG GTG ATT GAC CAC TGT GTG CCT
cys asp glu val ile asp his cys val pro

MATCH WITH FIG. 2I

TGC ATC CCC CTG GAC AAA GGA TTC AGC TGC
cys ile pro leu asp lys gly phe ser cys

TGT GAG ACA GAC AAT GAT GAC TGT GTG GCC
cys glu thr asp asn asp asp cys val ala

GAC ACA ATC AAT GGC TAC ACA TGC ACC TGC
asp thr ile asn gly tyr thr cys thr cys

CAC CCC CCA CCC ATG GTC CTA CTG CAG ACC
his pro pro pro met val leu leu gln thr

GGG GCC CAG TGC ATC GTG GTG CAG CAG GAG
gly ala gln cys ile val val gln gln glu

GGC CCC AGA TGC GAG AAG CTC ATC ACT GTC
gly pro arg cys glu lys leu ile thr val

CTG GCC TCC GCC AAG GTC CGA CCC CAG GCC
leu ala ser ala lys val arg pro gln ala

MATCH WITH FIG. 2L

FIG. 2K

MATCH WITH FIG. 2I

```
3760   AAC ATC TCC CTG CAG GTG GCC ACT GAC AAG
1181   asn ile ser leu gln val ala thr asp lys

3820   AAT GAC CCC CTG GCA CTG GAG CTG TAC CAG
1201   asn asp pro leu ala leu glu leu tyr gln

3880   AGT TCC CCT CCA ACC ACA GTG TAC AGT GTG
1221   ser ser pro pro thr thr val tyr ser val

3940   GTG GAG CTG GTG ACG CTA AAC CAG ACC CTG
1241   val glu leu val thr leu asn gln thr leu

4000   AGC CTG GGG AAG CTC CAG AAG CAG CCA GCA
1261   ser leu gly lys leu gln lys gln pro ala

4060   GGC ATC CCC ACC TCC ACC GGC CTC TCC GCC
1281   gly ile pro thr ser thr gly leu ser ala

4120   GGC TTC CAC GGA TGC ATC CAT GAG GTG CGC
1301   gly phe his gly cys ile his glu val arg

4180   CTC CCA CCA CAG TCC CTG GGG GTG TCA CCA
1321   leu pro pro gln ser leu gly val ser pro

4240   GGC CTG TGC CGC TCC GTG GAG AAG GAC AGC
1341   gly leu cys arg ser val glu lys asp ser

4300   GGC CCA CTC TGC GAC CAG GAG GCC CGG GAC
1361   gly pro leu cys asp gln glu ala arg asp

4360   AAA TGT GTG GCA ACT GGG ACC TCA TAC ATG
1381   lys cys val ala thr gly thr ser tyr met

4420   TTG TGT GAC AAC AAG AAT GAC TCT GCC AAT
1401   leu cys asp asn lys asn asp ser ala asn
```

MATCH WITH FIG. 2M

MATCH WITH FIG. 2L

FIG. 2L

MATCH WITH FIG. 2J

GAC AAC GGC ATC CTT CTC TAC AAA GGA GAC
asp asn gly ile leu leu tyr lys gly asp

GGC CAC GTG CGG CTG GTC TAT GAC AGC CTG
gly his val arg leu val tyr asp ser leu

GAG ACA GTG AAT GAT GGG CAG TTT CAC AGT
glu thr val asn asp gly gln phe his ser

AAC CTA GTA GTG GAC AAA GGA ACT CCA AAG
asn leu val val asp lys gly thr pro lys

MATCH WITH FIG. 2K

GTG GGC ATC AAC AGC CCC CTC TAC CTT GGA
val gly ile asn ser pro leu tyr leu gly

TTG CGC CAG GGC ACG GAC CGG CCT CTA GGC
leu arg gln gly thr asp arg pro leu gly

ATC AAC AAC GAG CTG CAG GAC TTC AAG GCC
ile asn asn glu leu gln asp phe lys ala

GGC TGC AAG TCC TGC ACC GTG TGC AAG CAC
gly cys lys ser cys thr val cys lys his

GTG GTG TGC GAG TGC CGC CCA GGC TGG ACC
val val cys glu cys arg pro gly trp thr

CCC TGC CTC GGC CAC AGA TGC CAC CAT GGA
pro cys leu gly his arg cys his his gly

TGC AAG TGT GCC GAG GGC TAT GGA GGG GAC
cys lys cys ala glu gly tyr gly gly asp

GCC TGC TCA GCC TTC AAG TGT CAC CAT GGG
ala cys ser ala phe lys cys his his gly

MATCH WITH FIG. 2N

FIG. 2M

MATCH WITH FIG. 2K

```
4480   CAG TGC CAC ATC TCA GAC CAA GGG GAG CCC
1421   gln cys his ile ser asp gln gly glu pro

4540   GAG CAC TGC CAA CAA GAG AAT CCG TGC CTG
1441   glu his cys gln gln glu asn pro cys leu

4600   CAG AAA GGT TAT GCA TCA TGT GCC ACA GCC
1461   gln lys gly tyr ala ser cys ala thr ala

4660   GGC TGT GGG CCC CAG TGC TGC CAG CCC ACC
1481   gly cys gly pro gln cys cys gln pro thr

4720   TGC ACG GAC GGC TCC TCG TTT GTA GAA GAG
1501   cys thr asp gly ser ser phe val glu glu

4780   GCG TGT TCC TAA GCCCCTGCCCGCCTGCCTGCCACCT
1521   ala cys ser Stop

4855   GGACCCCCTGGTGATTCAGCATGAAGGAAATGAAGCTGGAG
4934   AAATAAACAAAAAATAGAACTTATTTTTATTATGGAAAGTG
5013   TCTGCGTATATGTACCATATAGTGAGTTATTTTTACCAAGT
5092   TTTAAAAATTTAAGAAAAAAATAGACTAATAAAAATGCTTT
5171   GAGGAA
```

MATCH WITH FIG. 2N

FIG. 2N

MATCH WITH FIG. 2L

TAC TGC CTG TGC CAG CCC GGC TTT AGC GGC
tyr cys leu cys gln pro gly phe ser gly

GGA CAA GTA GTC CGA GAG GTG ATC CGC CGC
gly gln val val arg glu val ile arg arg

TCC AAG GTG CCC ATC ATG GAA TGT CGT GGG
ser lys val pro ile met glu cys arg gly

CGC AGC AAG CGG CGG AAA TAC GTC TTC CAG
arg ser lys arg arg lys tyr val phe gln

GTG GAG AGA CAC TTA GAG TGC GGC TGC CTC
val glu arg his leu glu cys gly cys leu

CTCGGACTCCAGCTTGATGGAGTTGGGACAGCCATGTG

AGGAAGGTAAAGAAGAAGAGAATATTAAGTATATTGTA
ACTATTTTCATCTTTTATTATATAAATATATTACACCA
TTTGTGTTGTGTATTTGTTGTGTTTTTAAAAATAGCTG
AAAACAAAAGGATAAGAATAAAGAATGATAGCCTGTCT

MATCH WITH FIG. 2M

FIG. 3A

```
hSlit   -MAPGWAGVG-AAVRARLALALALASVLSGPPAV-ACPTKCTCSAASVDCHGLGLR--AV 55
dSlit   MAAPSRTTLMPPPFRLQLRLLILPILLLLRHDAVHAEPYSGGFGSSAVSSGGLGSVGIHI 60
          **. : :  ...* :* * :    :*    ** * * .  .:::*.. ***     :

hSlit   PRG----I-----PRNAERLDLDRNNITR-ITKMDFAGLKNLRVLHLEDNQVSVIERGAF 105
dSlit   PGGGVGVITEARCPRVCSCTGLNVDCSHRGLTSVPRKISADVERLELQGNNLTVIYETDF 120
        * *    *     ** ..  .*: :   * :*.:      ::. *.*:.*:::** .  *

hSlit   QDLKQLERLRLNKNKLQVLPELLFQSTPKLTRLDLSENQIQGIPRKAFRGITDVKNLQLD 165
dSlit   QRLTKLRMLQLTDNQIHTIERNSFQDLVSLERLDISNNVITTVGRRVFKGAQSLRSLQLD 180
        * *.:*. *:*..*::::.: .  **.  .* ***:*:* *  : *:.*:*  .::.****

hSlit   NNHISCIEDGAFRALRDLEILTLNNNNISRILVTSFNHMPKIRTLRLHSNHLYCDCHLAW 225
dSlit   NNQITCLDEHAFKGLVELEILTLNNNNLTSLPHNIFGGLGRLRALRLSDNPFACDCHLSW 240
        **:*:*::: **:.* :**********:: :  . *. : ::*:*** .* : *****.*

hSlit   LSDWLRQRRTVGQFTLCMAPVHLRGFNVADVQKKEYVCP--APHSEPPSCNANSISCPSP 283
dSlit   LSRFLRSATRLAPYTRCQSPSQLKGQNVADLHDQEFKCSGLTEHA-PMECGA-ENSCPHP 298
        ** :**.   :. :* * :* :*:* ****::.:*: *.  : *: * .*.* . *** *

hSlit   CTCSNNIVDCRGKGLMEIPANLPEGIVEIRLEQNSIKAIPAGAFTQYKKLKRIDISKNQI 343
dSlit   CRCADGIVDCREKSLTSVPVTLPDDTTDVRLEQNFITELPPKSFSSFRRLRRIDLSNNNI 358
        * *::.***** *.* .:*..**:. .::***** *. :*. :*:.:::*:***:*:*:*
```

FIG. 3B

FIG. 3C

FIG. 3D

```
hSlit   CVPGYSGKLCETDN-DDCVAHKCRHGAQCVDTINGYTCTCPQGFSGPFCE-H-PPPMVLL1118
dSlit   CQAGFHGTNC-TDNIDDCQNHMCQNGGTCVDGINDYQCRCPDDYTGKYCEGHNMISMMYP1110
        * .*: *. * *** *** * *::*. *** **.* * **:.::* :** *   .*:

hSlit   QTSPCDQYECQNGAQCIV--VQ-QEPTCRCPPGFAGPRCEKLITVNFVGKDSYVELASAK1175
dSlit   QTSPCQNHECKHGV-CFQPNAQGSDYLCRCHPGYTGKWCEYLTSISFVHNNSFVELEPLR1169
        *****:::**::*. *:   .* .:  *** **::*  ** * ::.** ::*:*** . :

hSlit   VRPQANISLQVATDKDNGILLYKGDNDPLALELYQGHVRLVYDSLSSPPTTVYSVETVND1235
dSlit   TRPEANVTIVFSSAEQNGILMYDGQDAHLAVELFNGRIRVSYDVGNHPVSTMYSFEMVAD1229
        .**:**::: .:: ::****:*.*:: **:**::*::*: **  . * :*:**.* * *

hSlit   GQFHSVELVTLNQTLNLVVDKGTPKSLGKLQKQPAVGINSPLYLGGIPTSTGLSALRQGT1295
dSlit   GKYHAVELLAIKKNFTLRVDRGLARSIINEGSNDYLKLTTPMFLGGLPVDPAQQAYKNWQ1289
        *::*:***:::::::.* **:* .:*: :  .:  : :.:*::***:*.... .* ::

hSlit   DRPLGGFHGCIHEVRINNELQDFKALPPQSLGVSPGCKSCTVCKHGLCRSVEKDSVVCEC1355
dSlit   IRNLTSFKGCMKEVWINHKLVDFGNAQRQQK-ITPG---CALLE-GEQQEEEDD---EQD1341
        * * .*:**::** **::* **     *.  ::**   *:: : *  :. *.*    :
```

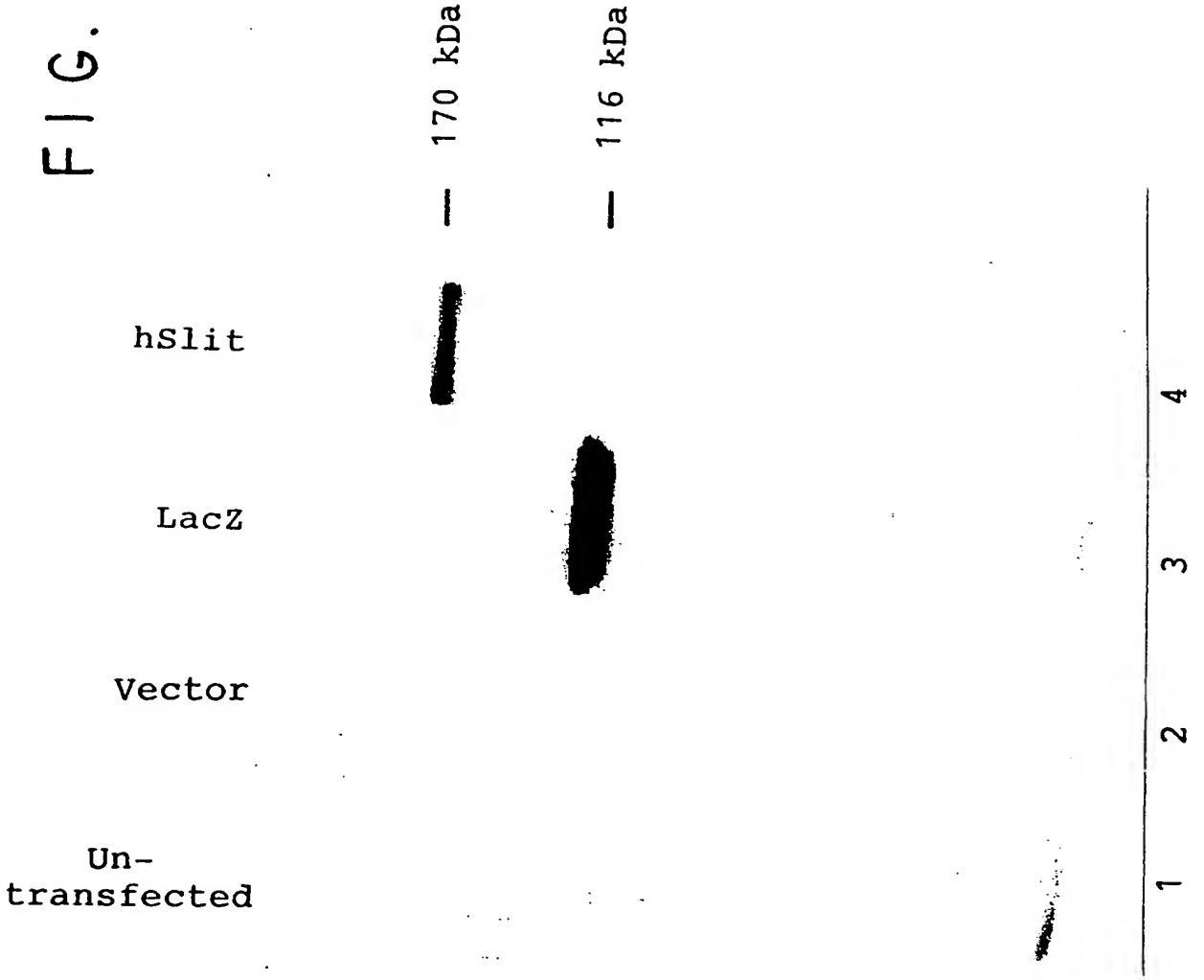
FIG. 3E

```
hSlit   RPGWTGPLCDQEARDPCLGHRCHHG-KCVATGTSYMCKCAEGYGGDLCDNKNDSANACSA1414
dSlit   FMDET-PHIKEEPVDPCLENKCRRGSRCVPNSNAR-----DGY----------QC--1380

hSlit   FKCHHGQCHISDQGEPYCLCQPGFSGEH-CQQENPCLGQVVREVIRRQKGYASCATASKV1473
dSlit   -KCKHGQ----RGR-YCDQGEGSTEPPTVTAASTCRKEQVREYY--TEN--DCRSRQPL1429

hSlit   PIMECRGGCGPQCCQPTRSKRRKYVFQCTDGSSFVEEVERHLECGCLA-CS 1523
dSlit   KYAKCVGGCGNQCCAAKIVRRRKVRMVCSNNRKYIKNLDIVRKCGCTKKCY 1480
```

FIG. 4



SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Timothy Connolly and Bhanu Rajput

(ii) TITLE OF INVENTION: Human MSC Slit and Polynucleotide Encoding Same

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN, CECCHI, STEWART & OLSTEIN
(B) STREET: 6 BECKER FARM ROAD
(C) CITY: ROSELAND
(D) STATE: NEW JERSEY
(E) COUNTRY: USA
(F) ZIP: 07068

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 INCH DISKETTE
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WORD PERFECT 5.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: concurrently
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: MULLINS, J.G.
(B) REGISTRATION NUMBER: 33,073
(C) REFERENCE/DOCKET NUMBER: 640100-236

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(A) TELEPHONE: 973-994-1700
(B) TELEFAX: 973-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 5176 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GCCGGCCCCG CCGATGGAGC TGCTGTTGCT GCCGCCGCCG CCTCCCGGAG          50
CGCCCCGCTC CGCCCGCGCC CCGTGCGCCT GAGCACCGAG CTCGCCCTCC         100
TCCGCCGCTA ACTCCGCCGC CCGCTCCCCA GGCCGCCCGC GCTCCCCGCG         150
CGCCTCCTCG GGCTCCACGC GTCTTGCCCC GCAGAGGCAG CCTCCTCCAG         200
GAGCGGGGCC CTGCACACC ATG GCC CCC GGG TGG GCA GGG GTC GGC       246
                       Met Ala Pro Gly Trp Ala Gly Val Gly
                         1               5

GCC GCC GTG CGC GCC CGC CTG GCG CTG GCC TTG GCG CTG GCG AGC    291
```

```
Ala Ala Val Arg Ala Arg Leu Ala Leu Ala Leu Ala Leu Ala Ser
10                  15                  20

GTC CTG AGT GGG CCT CCA GCC GTC GCC TGC CCC ACC AAG TGT ACC      336
Val Leu Ser Gly Pro Pro Ala Val Ala Cys Pro Thr Lys Cys Thr
25                  30                  35

TGC TCC GCT GCC AGC GTG GAC TGC CAC GGG CTG GGC CTC CGC GCG      381
Cys Ser Ala Ala Ser Val Asp Cys His Gly Leu Gly Leu Arg Ala
40                  45                  50

GTT CCT CGG GGC ATC CCC CGC AAC GCT GAG CGC CTT GAC CTG GAC      426
Val Pro Arg Gly Ile Pro Arg Asn Ala Glu Arg Leu Asp Leu Asp
 55                  60                  65
AGA AAT AAT ATC ACC AGG ATC ACC AAG ATG GAC TTC GCT GGG CTC      471
Arg Asn Asn Ile Thr Arg Ile Thr Lys Met Asp Phe Ala Gly Leu
 70                  75                  80

AAG AAC CTC CGA GTC TTG CAT CTG GAA GAC AAC CAG GTC AGC GTC      516
Lys Asn Leu Arg Val Leu His Leu Glu Asp Asn Gln Val Ser Val
 85                  90                  95

ATC GAG AGA GGC GCC TTC CAG GAC CTG AAG CAG CTA GAG CGA CTG      561
Ile Glu Arg Gly Ala Phe Gln Asp Leu Lys Gln Leu Glu Arg Leu
100                 105                 110

CGC CTG AAC AAG AAT AAG CTG CAA GTC CTT CCA GAA TTG CTT TTC      606
Arg Leu Asn Lys Asn Lys Leu Gln Val Leu Pro Glu Leu Leu Phe
115                 120                 125

CAG AGC ACG CCG AAG CTC ACC AGA CTA GAT TTG AGT GAA AAC CAG      651
Gln Ser Thr Pro Lys Leu Thr Arg Leu Asp Leu Ser Glu Asn Gln
130                 135                 140

ATC CAG GGG ATC CCG AGG AAG GCG TTC CGC GGC ATC ACC GAT GTG      696
Ile Gln Gly Ile Pro Arg Lys Ala Phe Arg Gly Ile Thr Asp Val
145                 150                 155

AAG AAC CTG CAA CTG GAC AAC AAC CAC ATC AGC TGC ATT GAA GAT      741
Lys Asn Leu Gln Leu Asp Asn Asn His Ile Ser Cys Ile Glu Asp
160                 165                 170

GGA GCC TTC CGA GCG CTG CGC GAT TTG GAG ATC CTT ACC CTC AAC      786
Gly Ala Phe Arg Ala Leu Arg Asp Leu Glu Ile Leu Thr Leu Asn
175                 180                 185

AAC AAC AAC ATC AGT CGC ATC CTG GTC ACC AGC TTC AAC CAC ATG      831
Asn Asn Asn Ile Ser Arg Ile Leu Val Thr Ser Phe Asn His Met
190                 195                 200

CCG AAG ATC CGA ACT CTG CGC CTC CAC TCC AAC CAC CTG TAC TGC      876
Pro Lys Ile Arg Thr Leu Arg Leu His Ser Asn His Leu Tyr Cys
205                 210                 215

GAC TGC CAC CTG GCC TGG CTC TCG GAT TGG CTG CGA CAG CGA CGG      921
Asp Cys His Leu Ala Trp Leu Ser Asp Trp Leu Arg Gln Arg Arg
220                 225                 230

ACA GTT GGC CAG TTC ACA CTC TGC ATG GCT CCT GTG CAT TTG AGG      966
Thr Val Gly Gln Phe Thr Leu Cys Met Ala Pro Val His Leu Arg
235                 240                 245

GGC TTC AAC GTG GCG GAT GTG CAG AAG AAG GAG TAC GTG TGC CCA     1011
Gly Phe Asn Val Ala Asp Val Gln Lys Lys Glu Tyr Val Cys Pro
250                 255                 260

GCC CCC CAC TCG GAG CCC CCA TCC TGC AAT GCC AAC TCC ATC TCC     1056
Ala Pro His Ser Glu Pro Pro Ser Cys Asn Ala Asn Ser Ile Ser
```

```
265                 270                 275

TGC CCT TCG CCC TGC ACG TGC AGC AAT AAC ATC GTG GAC TGT CGA     1101
Cys Pro Ser Pro Cys Thr Cys Ser Asn Asn Ile Val Asp Cys Arg
280                 285                 290

GGA AAG GGC TTG ATG GAG ATT CCT GCC AAC TTG CCG GAG GGC ATC     1146
Gly Lys Gly Leu Met Glu Ile Pro Ala Asn Leu Pro Glu Gly Ile
295                 300                 305

GTC GAA ATA CGC CTA GAA CAG AAC TCC ATC AAA GCC ATC CCT GCA     1191
Val Glu Ile Arg Leu Glu Gln Asn Ser Ile Lys Ala Ile Pro Ala
310                 315                 320

GGA GCC TTC ACC CAG TAC AAG AAA CTG AAG CGA ATA GAC ATC AGC     1236
Gly Ala Phe Thr Gln Tyr Lys Lys Leu Lys Arg Ile Asp Ile Ser
325                 330                 335

AAG AAT CAG ATA TCG GAT ATT GCT CCA GAT GCC TTC CAG GGC CTG     1281
Lys Asn Gln Ile Ser Asp Ile Ala Pro Asp Ala Phe Gln Gly Leu
340                 345                 350

AAA TCA CTC ACA TCG CTG GTC CTG TAT GGG AAC AAG ATC ACC GAG     1326
Lys Ser Leu Thr Ser Leu Val Leu Tyr Gly Asn Lys Ile Thr Glu
355                 360                 365

ATT GCC AAG GGA CTG TTT GAT GGG CTG GTG TCC CTA CAG CTG CTC     1371
Ile Ala Lys Gly Leu Phe Asp Gly Leu Val Ser Leu Gln Leu Leu
370                 375                 380

CTC CTC AAT GCC AAC AAG ATC AAC TGC CTG CGG GTG AAC ACG TTT     1416
Leu Leu Asn Ala Asn Lys Ile Asn Cys Leu Arg Val Asn Thr Phe
385                 390                 395

CAG GAC CTG CAG AAC CTC AAC TTG CTC TCC CTG TAT GAC AAC AAG     1461
Gln Asp Leu Gln Asn Leu Asn Leu Leu Ser Leu Tyr Asp Asn Lys
400                 405                 410

CTG CAG ACC ATC AGC AAG GGG CTC TTC GCC CCT CTG CAG TCC ATC     1506
Leu Gln Thr Ile Ser Lys Gly Leu Phe Ala Pro Leu Gln Ser Ile
415                 420                 425

CAG ACA CTC CAC TTA GCC CAA AAC CCA TTT GTG TGC GAC TGC CAC     1551
Gln Thr Leu His Leu Ala Gln Asn Pro Phe Val Cys Asp Cys His
430                 435                 440

TTG AAG TGG CTG GCC GAC TAC CTC CAG GAC AAC CCC ATC GAG ACA     1596
Leu Lys Trp Leu Ala Asp Tyr Leu Gln Asp Asn Pro Ile Glu Thr
445                 450                 455

AGC GGG GCC CGC TGC AGC AGC CCG CGC CGA CTC GCC AAC AAG CGC     1641
Ser Gly Ala Arg Cys Ser Ser Pro Arg Arg Leu Ala Asn Lys Arg
460                 465                 470

ATC AGC CAG ATC AAG AGC AAG AAG TTC CGC TGC TCA GGC TCC GAG     1686
Ile Ser Gln Ile Lys Ser Lys Lys Phe Arg Cys Ser Gly Ser Glu
475                 480                 485

GAT TAC CGC AGC AGG TTC AGC AGC GAG TGC TTC ATG GAC CTC GTG     1731
Asp Tyr Arg Ser Arg Phe Ser Ser Glu Cys Phe Met Asp Leu Val
490                 495                 500

TGC CCC GAG AAG TGT CGC TGT GAG GGC ACG ATT GTG GAC TGC TCC     1776
Cys Pro Glu Lys Cys Arg Cys Glu Gly Thr Ile Val Asp Cys Ser
505                 510                 515

AAC CAG AAG CTG GTC CGC ATC CCA AGC CAC CTC CCT GAA TAT GTC     1821
Asn Gln Lys Leu Val Arg Ile Pro Ser His Leu Pro Glu Tyr Val
```

```
520                 525                 530

ACC GAC CTG CGA CTG AAT GAC AAT GAG GTA TCT GTT CTG GAG GCC     1866
Thr Asp Leu Arg Leu Asn Asp Asn Glu Val Ser Val Leu Glu Ala
535                 540                 545

ACT GGC ATC TTC AAG AAG TTG CCC AAC CTG CGG AAA ATA AAT CTG     1911
Thr Gly Ile Phe Lys Lys Leu Pro Asn Leu Arg Lys Ile Asn Leu
550                 555                 560

AGT AAC AAT AAG ATC AAG GAG GTG CGA GAG GGA GCT TTC GAT GGA     1956
Ser Asn Asn Lys Ile Lys Glu Val Arg Glu Gly Ala Phe Asp Gly
565                 570                 575

GCA GCC AGC GTG CAG GAG CTG ATG CTG ACA GGG AAC CAG CTG GAG     2001
Ala Ala Ser Val Gln Glu Leu Met Leu Thr Gly Asn Gln Leu Glu
580                 585                 590

ACC GTG CAC GGG CGC GTG TTC CGT GGC CTC AGT GGC CTC AAA ACC     2046
Thr Val His Gly Arg Val Phe Arg Gly Leu Ser Gly Leu Lys Thr
595                 600                 605

TTG ATG CTG AGG AGT AAC TTG ATC AGC TGT GTG AGT AAT GAC ACC     2091
Leu Met Leu Arg Ser Asn Leu Ile Ser Cys Val Ser Asn Asp Thr
610                 615                 620

TTT GCC GGC CTG AGT TCG GTG AGA CTG CTG TCC CTC TAT GAC AAT     2136
Phe Ala Gly Leu Ser Ser Val Arg Leu Leu Ser Leu Tyr Asp Asn
625                 630                 635

CGG ATC ACC ACC ATC ACC CCT GGG GCC TTC ACC ACG CTT GTC TCC     2181
Arg Ile Thr Thr Ile Thr Pro Gly Ala Phe Thr Thr Leu Val Ser
640                 645                 650

CCT GTC CAC CAT AAA CCT CCT GTC CAA CCC CTT CAA CTG CAA CTG     2226
Pro Val His His Lys Pro Pro Val Gln Pro Leu Gln Leu Gln Leu
655                 660                 665

CCA CTG GCC TGG CTC GGC AAG TGG TTG AGG AAG AGG CGG ATC GTC     2271
Pro Leu Ala Trp Leu Gly Lys Trp Leu Arg Lys Arg Arg Ile Val
670                 675                 680

AGT GGG AAC CCT AGG TGC CAG AAG CCA TTT TTC CTC AAG GAG ATC     2316
Ser Gly Asn Pro Arg Cys Gln Lys Pro Phe Phe Leu Lys Glu Ile
685                 690                 695

CCC ATC CAG GAT GTG GCC ATC CAG GAC TTC ACC TGT GAT GGC AAC     2361
Pro Ile Gln Asp Val Ala Ile Gln Asp Phe Thr Cys Asp Gly Asn
700                 705                 710

GAG GAG AGT AGT TGC CAG CTG AGC CCG CGC TGC CCG GAG CAG TGC     2406
Glu Glu Ser Ser Cys Gln Leu Ser Pro Arg Cys Pro Glu Gln Cys
715                 720                 725

ACC TGT ATG GAG ACA GTG GTG CGA TGC AGC AAC AAG GGG CTC CGC     2451
Thr Cys Met Glu Thr Val Val Arg Cys Ser Asn Lys Gly Leu Arg
730                 735                 740

GCC CTC CCC AGA GGC ATG CCC AAG GAT GTG ACC GAG CTG TAC CTG     2496
Ala Leu Pro Arg Gly Met Pro Lys Asp Val Thr Glu Leu Tyr Leu
745                 750                 755

GAA GGA AAC CAC CTA ACA GCC GTG CCC AGA GAG CTG TCC GCC CTC     2541
Glu Gly Asn His Leu Thr Ala Val Pro Arg Glu Leu Ser Ala Leu
760                 765                 770

CGA CAC CTG ACG CTT ATT GAC CTG AGC AAC AAC AGC ATC AGC ATG     2586
Arg His Leu Thr Leu Ile Asp Leu Ser Asn Asn Ser Ile Ser Met
```

```
775                 780                 785

CTG ACC AAT TAC ACC TTC AGT AAC ATG TCT CAC CTC TCC ACT CTG      2631
Leu Thr Asn Tyr Thr Phe Ser Asn Met Ser His Leu Ser Thr Leu
790                 795                 800

ATC CTG AGC TAC AAC CGG CTG AGG TGC ATC CCC GTC CAC GCC TTC      2676
Ile Leu Ser Tyr Asn Arg Leu Arg Cys Ile Pro Val His Ala Phe
805                 810                 815

AAC GGG CTG CGG TCC CTG CGA GTG CTA ACC CTC CAT GGC AAT GAC      2721
Asn Gly Leu Arg Ser Leu Arg Val Leu Thr Leu His Gly Asn Asp
820                 825                 830

ATT TCC AGC GTT CCT GAA GGC TCC TTC AAC GAC CTC ACA TCT CTT      2766
Ile Ser Ser Val Pro Glu Gly Ser Phe Asn Asp Leu Thr Ser Leu
835                 840                 845

TCC CAT CTG GCG CTG GGA ACC AAC CCA CTC CAC TGT GAC TGC AGT      2811
Ser His Leu Ala Leu Gly Thr Asn Pro Leu His Cys Asp Cys Ser
850                 855                 860

CTT CGG TGG CTG TCG GAG TGG GTG AAG GCG GGG TAC AAG GAG CCT      2856
Leu Arg Trp Leu Ser Glu Trp Val Lys Ala Gly Tyr Lys Glu Pro
865                 870                 875

GGC ATC GCC CGC TGC AGT AGC CCT GAG CCC ATG GCT GAC AGG CTC      2901
Gly Ile Ala Arg Cys Ser Ser Pro Glu Pro Met Ala Asp Arg Leu
880                 885                 890

CTG CTC ACC ACC CCA ACC CAC CGC TTC CAG TGC AAA GGG CCA GTG      2946
Leu Leu Thr Thr Pro Thr His Arg Phe Gln Cys Lys Gly Pro Val
895                 900                 905

GAC ATC AAC ATT GTG GCC AAA TGC AAT GCC TGC CTC TCC AGC CCG      2991
Asp Ile Asn Ile Val Ala Lys Cys Asn Ala Cys Leu Ser Ser Pro
910                 915                 920

TGC AAG AAT AAC GGG ACA TGC ACC CAG GAC CCT GTG GAG CTG TAC      3036
Cys Lys Asn Asn Gly Thr Cys Thr Gln Asp Pro Val Glu Leu Tyr
925                 930                 935

CGC TGT GCC TGC CCC TAC AGC TAC AAG GGC AAG GAC TGC ACT GTG      3081
Arg Cys Ala Cys Pro Tyr Ser Tyr Lys Gly Lys Asp Cys Thr Val
940                 945                 950

CCC ATC AAC ACC TGC ATC CAG AAC CCC TGT CAG CAT GGA GGC ACC      3126
Pro Ile Asn Thr Cys Ile Gln Asn Pro Cys Gln His Gly Gly Thr
955                 960                 965

TGC CAC CTG AGT GAC AGC CAC AAG GAT GGG TTC AGC TGC TCC TGC      3171
Cys His Leu Ser Asp Ser His Lys Asp Gly Phe Ser Cys Ser Cys
970                 975                 980

CCT CTG GGC TTT GAG GGG CAG CGG TGT GAG ATC AAC CCA GAT GAC      3216
Pro Leu Gly Phe Glu Gly Gln Arg Cys Glu Ile Asn Pro Asp Asp
985                 990                 995

TGT GAG GAC AAC GAC TGC GAA AAC AAT GCC ACC TGC GTG GAC GGG      3261
Cys Glu Asp Asn Asp Cys Glu Asn Asn Ala Thr Cys Val Asp Gly
1000                1005                1010

ATC AAC AAC TAC GTG TGT ATC TGT CCG CCT AAC TAC ACA GGT GAG      3306
Ile Asn Asn Tyr Val Cys Ile Cys Pro Pro Asn Tyr Thr Gly Glu
1015                1020                1025

CTA TGC GAC GAG GTG ATT GAC CAC TGT GTG CCT GAG CTG AAC CTC      3351
Leu Cys Asp Glu Val Ile Asp His Cys Val Pro Glu Leu Asn Leu
```

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1030                1035                1040

TGT CAG CAT GAG GCC AAG TGC ATC CCC CTG GAC AAA GGA TTC AGC     3396
Cys Gln His Glu Ala Lys Cys Ile Pro Leu Asp Lys Gly Phe Ser
1045                1050                1055

TGC GAG TGT GTC CCT GGC TAC AGC GGG AAG CTC TGT GAG ACA GAC     3441
Cys Glu Cys Val Pro Gly Tyr Ser Gly Lys Leu Cys Glu Thr Asp
1060                1065                1070

AAT GAT GAC TGT GTG GCC CAC AAG TGC CGC CAC GGG GCC CAG TGC     3486
Asn Asp Asp Cys Val Ala His Lys Cys Arg His Gly Ala Gln Cys
1075                1080                1085

GTG GAC ACA ATC AAT GGC TAC ACA TGC ACC TGC CCC CAG GGC TTC     3531
Val Asp Thr Ile Asn Gly Tyr Thr Cys Thr Cys Pro Gln Gly Phe
1090                1095                1100

AGT GGA CCC TTC TGT GAA CAC CCC CCA CCC ATG GTC CTA CTG CAG     3576
Ser Gly Pro Phe Cys Glu His Pro Pro Pro Met Val Leu Leu Gln
1105                1110                1115

ACC AGC CCA TGC GAC CAG TAC GAG TGC CAG AAC GGG GCC CAG TGC     3621
Thr Ser Pro Cys Asp Gln Tyr Glu Cys Gln Asn Gly Ala Gln Cys
1120                1125                1130

ATC GTG GTG CAG CAG GAG CCC ACC TGC CGC TGC CCA CCA GGC TTC     3666
Ile Val Val Gln Gln Glu Pro Thr Cys Arg Cys Pro Pro Gly Phe
1135                1140                1145

GCC GGC CCC AGA TGC GAG AAG CTC ATC ACT GTC AAC TTC GTG GGC     3711
Ala Gly Pro Arg Cys Glu Lys Leu Ile Thr Val Asn Phe Val Gly
1150                1155                1160

AAA GAC TCC TAC GTG GAA CTG GCC TCC GCC AAG GTC CGA CCC CAG     3756
Lys Asp Ser Tyr Val Glu Leu Ala Ser Ala Lys Val Arg Pro Gln
1165                1170                1175

GCC AAC ATC TCC CTG CAG GTG GCC ACT GAC AAG GAC AAC GGC ATC     3801
Ala Asn Ile Ser Leu Gln Val Ala Thr Asp Lys Asp Asn Gly Ile
1180                1185                1190

CTT CTC TAC AAA GGA GAC AAT GAC CCC CTG GCA CTG GAG CTG TAC     3846
Leu Leu Tyr Lys Gly Asp Asn Asp Pro Leu Ala Leu Glu Leu Tyr
1195                1200                1205

CAG GGC CAC GTG CGG CTG GTC TAT GAC AGC GTG AGT TCC CCT CCA     3891
Gln Gly His Val Arg Leu Val Tyr Asp Ser Val Ser Ser Pro Pro
1210                1215                1220

ACC ACA GTG TAC AGT GTG GAG ACA GTG AAT GAT GGG CAG TTT CAC     3936
Thr Thr Val Tyr Ser Val Glu Thr Val Asn Asp Gly Gln Phe His
1225                1230                1235

AGT GTG GAG GTG GTG ACG CTA AAC CAG ACC CTG AAC TTA GTA GTG     3981
Ser Val Glu Val Val Thr Leu Asn Gln Thr Leu Asn Leu Val Val
1240                1245                1250

GAC AAA GGA ACT CCA AAG AGC TTG GGG AAG TTC CAG AAG CAG CCA     4026
Asp Lys Gly Thr Pro Lys Ser Leu Gly Lys Phe Gln Lys Gln Pro
1255                1260                1265

GCA GTG GGC ATC AAC AGC CCC CTC TAC CTT GGA GGC ATC CCC ACC     4071
Ala Val Gly Ile Asn Ser Pro Leu Tyr Leu Gly Gly Ile Pro Thr
1270                1275                1280

TCC ACC GGC CTC TCC GCC TTG CGC CAG GGC ACG GAC CGG CCT CTA     4116
Ser Thr Gly Leu Ser Ala Leu Arg Gln Gly Thr Asp Arg Pro Leu
```

```
1285                1290                1295

GGC GGC TTC CAC GGA TGC ATC CAT GAG GTG CGC ATC AAC AAC GAG      4161
Gly Gly Phe His Gly Cys Ile His Glu Val Arg Ile Asn Asn Glu
1300                1305                1310

CTG CAG GAC TTC AAG GCC CTC CCA CCA CAG TCC CTG GGG GTG TCA      4206
Leu Gln Asp Phe Lys Ala Leu Pro Pro Gln Ser Leu Gly Val Ser
1315                1320                1325

CCA GGC TGC AAG TCC TGC ACC GTG TGC AAG CAC GGC CTG TGC CGC      4251
Pro Gly Cys Lys Ser Cys Thr Val Cys Lys His Gly Leu Cys Arg
1330                1335                1340

TCC GTG GAG AAG GAC AGC GTG GTG TGC GAG TGC CGC CCA GGC TGG      4296
Ser Val Glu Lys Asp Ser Val Val Cys Glu Cys Arg Pro Gly Trp
1345                1350                1355

ACC GGC CCA CTC TGC GAT CAG GAG GCC CGG GAC CCC TGC CTC GGC      4341
Thr Gly Pro Leu Cys Asp Gln Glu Ala Arg Asp Pro Cys Leu Gly
1360                1365                1370

CAC AGA TGC CAC CAT GGA AAA TGT GTG GCA ACT GGG ACC TCA TAC      4386
His Arg Cys His His Gly Lys Cys Val Ala Thr Gly Thr Ser Tyr
1375                1380                1385

ATG TGC AAG TGT GCC GAG GGC TAT GGA GGG GAC TTG TGT GAC AAC      4431
Met Cys Lys Cys Ala Glu Gly Tyr Gly Gly Asp Leu Cys Asp Asn
1390                1395                1400

AAG AAT GAC TCT GCC AAT GCC TGC TCA GCC TTC AAG TGT CAC CAT      4476
Lys Asn Asp Ser Ala Asn Ala Cys Ser Ala Phe Lys Cys His His
1405                1410                1415

GGG CAG TGC CAC ATC TCA GAC CAA GGG GAG CCC TAC TGC CTG TGC      4521
Gly Gln Cys His Ile Ser Asp Gln Gly Glu Pro Tyr Cys Leu Cys
1420                1425                1430

CAG CCC GGC TTT AGC GGC GAG CAC TGC CAA CAA GAG AAT CCG TGC      4566
Gln Pro Gly Phe Ser Gly Glu His Cys Gln Gln Glu Asn Pro Cys
1435                1440                1445

CTG GGA CCA GTA GTC CGA GAG GTG ATC CGC CGC CAG AAA GGT TAT      4611
Leu Gly Gln Val Val Arg Glu Val Ile Arg Arg Gln Lys Gly Tyr
1450                1455                1460

GCA TCA TGT GCC ACA GCC TCC AAG GTG CCC ATC ATG GAA TGT CGT      4656
Ala Ser Cys Ala Thr Ala Ser Lys Val Pro Ile Met Glu Cys Arg
1465                1470                1475

GGG GGC TGT GGG CCC CAG TGC TGC CAG CCC ACC CGC AGC AAG CGG      4701
Gly Gly Cys Gly Pro Gln Cys Cys Gln Pro Thr Arg Ser Lys Arg
1480                1485                1490

CGG AAA TAC GTC TTC CAG TGC ACG GAC GGC TCC TCG TTT GTA GAA      4746
Arg Lys Tyr Val Phe Gln Cys Thr Asp Gly Ser Ser Phe Val Glu
1495                1500                1505

GAG GTG GAG AGA CAG TTA GAG TGC GGC TGC CTC GCG TGT TCC TAA      4791
Glu Val Glu Arg His Leu Glu Cys Gly Cys Leu Ala Cys Ser
1510                1515                1520

GCCCCTGCCC GCCTGCCTGC CACCTCTCGG ACTCCAGCTT GATGGAGTTG          4841

GGACAGCCAT GTGGGACCCC CGGGTGATTC AGCATGAAGG AAATGAAGCT          4891

GGAGAGGAAG GTAAAGAAGA AGAGAATATT AAGTATATTG TAAAATAAAC          4941
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AAAAAATAGA ACTTATTTTT ATTATGGAAA GTGACTATTT TCATCTTTTA          4991

TTATATAAAT ATATTACACC ATCTGCGTAT ATGTACCATA TAGTGAGTTA          5041

TTTTTACCAA GTTTTGTGTT GTGTATTTGT TGTGTTTTTA AAAATAGCTG          5091

TTTAAAAATT TAAGAAAAAA ATAGACTAAT AAAAATGCTT TAAAACAAAA          5141

GGATAAGAAT AAAGAATGAT AGCCTGTCTG AGGAA                          5176
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 1523 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ala Pro Gly Trp Ala Gly Val Gly Ala Ala Val Arg Ala Arg
  5                  10                  15
Leu Ala Leu Ala Leu Ala Leu Ala Ser Val Leu Ser Gly Pro Pro
 20                  25                  30
Ala Val Ala Cys Pro Thr Lys Cys Thr Cys Ser Ala Ala Ser Val
 35                  40                  45
Asp Cys His Gly Leu Gly Leu Arg Ala Val Pro Arg Gly Ile Pro
 50                  55                  60
Arg Asn Ala Glu Arg Leu Asp Leu Asp Arg Asn Asn Ile Thr Arg
 65                  70                  75
Ile Thr Lys Met Asp Phe Ala Gly Leu Lys Asn Leu Arg Val Leu
 80                  85                  90
His Leu Glu Asp Asn Gln Val Ser Val Ile Glu Arg Gly Ala Phe
 95                 100                 105
Gln Asp Leu Lys Gln Leu Glu Arg Leu Arg Leu Asn Lys Asn Lys
110                 115                 120
Leu Gln Val Leu Pro Glu Leu Leu Phe Gln Ser Thr Pro Lys Leu
125                 130                 135
Thr Arg Leu Asp Leu Ser Glu Asn Gln Ile Gln Gly Ile Pro Arg
140                 145                 150
Lys Ala Phe Arg Gly Ile Thr Asp Val Lys Asn Leu Gln Leu Asp
155                 160                 165
Asn Asn His Ile Ser Cys Ile Glu Asp Gly Ala Phe Arg Ala Leu
170                 175                 180
Arg Asp Leu Glu Ile Leu Thr Leu Asn Asn Asn Asn Ile Ser Arg
185                 190                 195
Ile Leu Val Thr Ser Phe Asn His Met Pro Lys Ile Arg Thr Leu
200                 205                 210
Arg Leu His Ser Asn His Leu Tyr Cys Asp Cys His Leu Ala Trp
215                 220                 225
Leu Ser Asp Trp Leu Arg Gln Arg Arg Thr Val Gly Gln Phe Thr
230                 235                 240
Leu Cys Met Ala Pro Val His Leu Arg Gly Phe Asn Val Ala Asp
245                 250                 255
Val Gln Lys Lys Glu Tyr Val Cys Pro Ala Pro His Ser Glu Pro
260                 265                 270
Pro Ser Cys Asn Ala Asn Ser Ile Ser Cys Pro Ser Pro Cys Thr
275                 280                 285
Cys Ser Asn Asn Ile Val Asp Cys Arg Gly Lys Gly Leu Met Glu
290                 295                 300
Ile Pro Ala Asn Leu Pro Glu Gly Ile Val Glu Ile Arg Leu Glu
305                 310                 315
Gln Asn Ser Ile Lys Ala Ile Pro Ala Gly Ala Phe Thr Gln Tyr
320                 325                 330
Lys Lys Leu Lys Arg Ile Asp Ile Ser Lys Asn Gln Ile Ser Asp
335                 340                 345
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Ile Ala Pro Asp Ala Phe Gln Gly Leu Lys Ser Leu Thr Ser Leu
                350                 355                 360
Val Leu Tyr Gly Asn Lys Ile Thr Glu Ile Ala Lys Gly Leu Phe
                365                 370                 375

Asp Gly Leu Val Ser Leu Gln Leu Leu Leu Leu Asn Ala Asn Lys
                380                 385                 390
Ile Asn Cys Leu Arg Val Asn Thr Phe Gln Asp Leu Gln Asn Leu
                395                 400                 405
Asn Leu Leu Ser Leu Tyr Asp Asn Lys Leu Gln Thr Ile Ser Lys
                410                 415                 420
Gly Leu Phe Ala Pro Leu Gln Ser Ile Gln Thr Leu His Leu Ala
                425                 430                 435
Gln Asn Pro Phe Val Cys Asp Cys His Leu Lys Trp Leu Ala Asp
                440                 445                 450
Tyr Leu Gln Asp Asn Pro Ile Glu Thr Ser Gly Ala Arg Cys Ser
                455                 460                 465
Ser Pro Arg Arg Leu Ala Asn Lys Arg Ile Ser Gln Ile Lys Ser
                470                 475                 480
Lys Lys Phe Arg Cys Ser Gly Ser Glu Asp Tyr Arg Ser Arg Phe
                485                 490                 495
Ser Ser Glu Cys Phe Met Asp Leu Val Cys Pro Glu Lys Cys Arg
                500                 505                 510
Cys Glu Gly Thr Ile Val Asp Cys Ser Asn Gln Lys Leu Val Arg
                515                 520                 525
Ile Pro Ser His Leu Pro Glu Tyr Val Thr Asp Leu Arg Leu Asn
                530                 535                 540
Asp Asn Glu Val Ser Val Leu Glu Ala Thr Gly Ile Phe Lys Lys
                545                 550                 555
Leu Pro Asn Leu Arg Lys Ile Asn Leu Ser Asn Asn Lys Ile Lys
                560                 565                 570
Glu Val Arg Glu Gly Ala Phe Asp Gly Ala Ala Ser Val Gln Glu
                575                 580                 585
Leu Met Leu Thr Gly Asn Gln Leu Glu Thr Val His Gly Arg Val
                590                 595                 600
Phe Arg Gly Leu Ser Gly Leu Lys Thr Leu Met Leu Arg Ser Asn
                605                 610                 615
Leu Ile Ser Cys Val Ser Asn Asp Thr Phe Ala Gly Leu Ser Ser
                620                 625                 630
Val Arg Leu Leu Ser Leu Tyr Asp Asn Arg Ile Thr Thr Ile Thr
                635                 640                 645
Pro Gly Ala Phe Thr Thr Leu Val Ser Pro Val His His Lys Pro
                650                 655                 660
Pro Val Gln Pro Leu Gln Leu Gln Leu Pro Leu Ala Trp Leu Gly
                665                 670                 675
Lys Trp Leu Arg Lys Arg Arg Ile Val Ser Gly Asn Pro Arg Cys
                680                 685                 690
Gln Lys Pro Phe Phe Leu Lys Glu Ile Pro Ile Gln Asp Val Ala
                695                 700                 705
Ile Gln Asp Phe Thr Cys Asp Gly Asn Glu Glu Ser Ser Cys Gln
                710                 715                 720
Leu Ser Pro Arg Cys Pro Glu Gln Cys Thr Cys Met Glu Thr Val
                725                 730                 735
Val Arg Cys Ser Asn Lys Gly Leu Arg Ala Leu Pro Arg Gly Met
                740                 745                 750
Pro Lys Asp Val Thr Glu Leu Tyr Leu Glu Gly Asn His Leu Thr
                755                 760                 765

Ala Val Pro Arg Glu Leu Ser Ala Leu Arg His Leu Thr Leu Ile
                770                 775                 780
Asp Leu Ser Asn Asn Ser Ile Ser Met Leu Thr Asn Tyr Thr Phe
                785                 790                 795
Ser Asn Met Ser His Leu Ser Thr Leu Ile Leu Ser Tyr Asn Arg
                800                 805                 810
Leu Arg Cys Ile Pro Val His Ala Phe Asn Gly Leu Arg Ser Leu
                815                 820                 825
Arg Val Leu Thr Leu His Gly Asn Asp Ile Ser Ser Val Pro Glu
                830                 835                 840
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Gly Ser Phe Asn Asp Leu Thr Ser Leu Ser His Leu Ala Leu Gly
                845                 850                 855
Thr Asn Pro Leu His Cys Asp Cys Ser Leu Arg Trp Leu Ser Glu
                860                 865                 870
Trp Val Lys Ala Gly Tyr Lys Glu Pro Gly Ile Ala Arg Cys Ser
                875                 880                 885
Ser Pro Glu Pro Met Ala Asp Arg Leu Leu Leu Thr Thr Pro Thr
                890                 895                 900
His Arg Phe Gln Cys Lys Gly Pro Val Asp Ile Asn Ile Val Ala
                905                 910                 915
Lys Cys Asn Ala Cys Leu Ser Ser Pro Cys Lys Asn Asn Gly Thr
                920                 925                 930
Cys Thr Gln Asp Pro Val Glu Leu Tyr Arg Cys Ala Cys Pro Tyr
                935                 940                 945
Ser Tyr Lys Gly Lys Asp Cys Thr Val Pro Ile Asn Thr Cys Ile
                950                 955                 960
Gln Asn Pro Cys Gln His Gly Gly Thr Cys His Leu Ser Asp Ser
                965                 970                 975
His Lys Asp Gly Phe Ser Cys Ser Cys Pro Leu Gly Phe Glu Gly
                980                 985                 990
Gln Arg Cys Glu Ile Asn Pro Asp Asp Cys Glu Asp Asn Asp Cys
                995                1000                1005
Glu Asn Asn Ala Thr Cys Val Asp Gly Ile Asn Asn Tyr Val Cys
               1010                1015                1020
Ile Cys Pro Pro Asn Tyr Thr Gly Glu Leu Cys Asp Glu Val Ile
               1025                1030                1035
Asp His Cys Val Pro Glu Leu Asn Leu Cys Gln His Glu Ala Lys
               1040                1045                1050
Cys Ile Pro Leu Asp Lys Gly Phe Ser Cys Glu Cys Val Pro Gly
               1055                1060                1065
Tyr Ser Gly Lys Leu Cys Glu Thr Asp Asn Asp Asp Cys Val Ala
               1070                1075                1080
His Lys Cys Arg His Gly Ala Gln Cys Val Asp Thr Ile Asn Gly
               1085                1090                1095
Tyr Thr Cys Thr Cys Pro Gln Gly Phe Ser Gly Pro Phe Cys Glu
               1100                1105                1110
His Pro Pro Pro Met Val Leu Leu Gln Thr Ser Pro Cys Asp Gln
               1115                1120                1125
Tyr Glu Cys Gln Asn Gly Ala Gln Cys Ile Val Val Gln Gln Glu
               1130                1135                1140
Pro Thr Cys Arg Cys Pro Pro Gly Phe Ala Gly Pro Arg Cys Glu
               1145                1150                1155

Lys Leu Ile Thr Val Asn Phe Val Gly Lys Asp Ser Tyr Val Glu
               1160                1165                1170
Leu Ala Ser Ala Lys Val Arg Pro Gln Ala Asn Ile Ser Leu Gln
               1175                1180                1185
Val Ala Thr Asp Lys Asp Asn Gly Ile Leu Leu Tyr Lys Gly Asp
               1190                1195                1200
Asn Asp Pro Leu Ala Leu Glu Leu Tyr Gln Gly His Val Arg Leu
               1205                1210                1215
Val Tyr Asp Ser Val Ser Ser Pro Pro Thr Thr Val Tyr Ser Val
               1220                1225                1230
Glu Thr Val Asn Asp Gly Gln Phe His Ser Val Glu Val Val Thr
               1235                1240                1245
Leu Asn Gln Thr Leu Asn Leu Val Val Asp Lys Gly Thr Pro Lys
               1250                1255                1260
Ser Leu Gly Lys Phe Gln Lys Gln Pro Ala Val Gly Ile Asn Ser
               1265                1270                1275
Pro Leu Tyr Leu Gly Gly Ile Pro Thr Ser Thr Gly Leu Ser Ala
               1280                1285                1290
Leu Arg Gln Gly Thr Asp Arg Pro Leu Gly Gly Phe His Gly Cys
               1295                1300                1305
Ile His Glu Val Arg Ile Asn Asn Glu Leu Gln Asp Phe Lys Ala
               1310                1315                1320
Leu Pro Pro Gln Ser Leu Gly Val Ser Pro Gly Cys Lys Ser Cys
               1325                1330                1335
Thr Val Cys Lys His Gly Leu Cys Arg Ser Val Glu Lys Asp Ser
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                1340                1345                1350
Val Val Cys Glu Cys Arg Pro Gly Trp Thr Gly Pro Leu Cys Asp
                1355                1360                1365
Gln Glu Ala Arg Asp Pro Cys Leu Gly His Arg Cys His His Gly
                1370                1375                1380
Lys Cys Val Ala Thr Gly Thr Ser Tyr Met Cys Lys Cys Ala Glu
                1385                1390                1395
Gly Tyr Gly Gly Asp Leu Cys Asp Asn Lys Asn Asp Ser Ala Asn
                1400                1405                1410
Ala Cys Ser Ala Phe Lys Cys His His Gly Gln Cys His Ile Ser
                1415                1420                1425
Asp Gln Gly Glu Pro Tyr Cys Leu Cys Gln Pro Gly Phe Ser Gly
                1430                1435                1440
Glu His Cys Gln Gln Glu Asn Pro Cys Leu Gly Gln Val Val Arg
                1445                1450                1455
Glu Val Ile Arg Arg Gln Lys Gly Tyr Ala Ser Cys Ala Thr Ala
                1460                1465                1470
Ser Lys Val Pro Ile Met Glu Cys Arg Gly Gly Cys Gly Pro Gln
                1475                1480                1485
Cys Cys Gln Pro Thr Arg Ser Lys Arg Arg Lys Tyr Val Phe Gln
                1490                1495                1500
Cys Thr Asp Gly Ser Ser Phe Val Glu Glu Val Glu Arg His Leu
                1505                1510                1515
Glu Cys Gly Cys Leu Ala Cys Ser
                1520
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 NUCLEOTIDES
(B) TYPE: DNA
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: OLIGONUCLEOTIDE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

(2) INFORMATION FOR SEQ ID NO:3:

TCCTCGGGCT CCACGCGTCT T 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 NUCLEOTIDES
(B) TYPE: DNA
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: OLIGONUCLEOTIDE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGTACATATA CGCAGATGGT G 21

INTERNATIONAL SEARCH REPORT

Int. .tional Application No
PCT/US 98/22845

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N15/11 C12N5/10 C12Q1/68 C07K14/47
C07K16/18 A61K38/17 A61K39/395 A61K48/00 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N C12Q A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HILLIER L. ET AL.: "The WashU-Merck EST Project 1997, AC AA496230" EMBL DATABSE,3 July 1997, XP002097995 Heidelberg see the whole document ---	2
X	"AC AA506521" EMBL DATABASE,4 July 1997, XP002097996 Heidelberg see the whole document ---	2
A	WO 92 10518 A (UNIV YALE ;ROTHBERG JONATHAN MARC (US); ARTAVANIS TSAKONAS SPYRIDO) 25 June 1992 see the whole document ---	1-20
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 March 1999	12/04/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

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INTERNATIONAL SEARCH REPORT

Int ational Application No
PCT/US 98/22845

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	ROTHBERG J. ET AL.: "slit: an extracellular protein necessary for development of midline glia and commissural axon pathways contains both EGF and LRR domains" GENES & DEVELOPMENT, vol. 4, no. 12a, December 1990, pages 2169-2187, XP002097997 cited in the application see the whole document	1-20
P,X	NAKAYAMA M ET AL: "Identification of high-molecular-weight proteins with multiple EGF-like motifs by motif-trap screening." GENOMICS, (1998 JUL 1) 51 (1) 27-34. JOURNAL CODE: GEN. ISSN: 0888-7543., XP002097998 United States see the whole document	1-3,5-7, 11,13
T	DATABASE WPI Section Ch, Week 9824 Derwent Publications Ltd., London, GB; Class C07,Page 45, AN 98-267127 XP002097999 & JP 10 087699 A (ASAHI KASEI KOGYO KK) , 7 April 1998 see abstract	1-20

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/ 22845

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 15-18 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
See FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. tional Application No
PCT/US 98/22845

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9210518 A	25-06-1992	NONE	